Procedure in
English
German
French
NEW!

Plasmid DNA Purification

User manual

NucleoBond® Xtra Midi

NucleoBond® Xtra Maxi

NucleoBond® Xtra Midi Plus

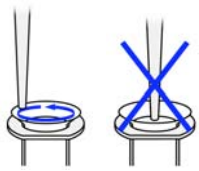
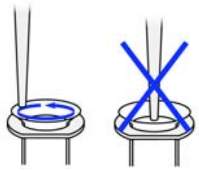
NucleoBond® Xtra Maxi Plus

March 2006/Rev. 01

MACHEREY-NAGEL

Protocol at a glance (Rev. 01)

Plasmid DNA Purification (NucleoBond® Xtra Midi / Maxi)

	Midi	Maxi
1 Cultivate and harvest bacterial cells	4,500-6,000 x g 15 min at 4°C	
2 Cell lysis	high-copy / low-copy	high-copy / low-copy
Buffer RES	8 ml / 16 ml	12 ml / 24 ml
Buffer LYS	8 ml / 16 ml	12 ml / 24 ml
3 Equilibration of the column together with inserted column filter	Buffer EQU 12 ml	 Buffer EQU 25 ml
4 Neutralization	Buffer NEU 8 ml / 16 ml	Buffer NEU 12 ml / 24 ml
5 Clarification and loading of the lysate	invert the tube 3 times load lysate on NucleoBond® Xtra column filter lysate is simultaneously cleared and loaded onto the NucleoBond® Xtra column	
6 1st Washing	**Buffer EQU** 5 ml !	 **Buffer EQU** 15 ml !
7 Discard NucleoBond® Xtra column filter	discard NucleoBond® Xtra column filter	discard NucleoBond® Xtra column filter
8 2nd Washing	**Buffer WASH** 8 ml !	**Buffer WASH** 25 ml !
9 Elution	Buffer ELU 5 ml	Buffer ELU 15 ml

10 Precipitation	**NucleoBond® Xtra Midi**	**NucleoBond® Xtra Midi Plus**	**NucleoBond® Xtra Maxi**	**NucleoBond® Xtra Maxi Plus**
	Isopropanol 3.5 ml 15,000 x g 30 min at 4°C	Isopropanol 3.5 ml load NucleoBond® Finalizer	Isopropanol 10.5 ml 15,000 x g 30 min at 4°C	Isopropanol 10.5 ml load NucleoBond® Finalizer Large
11 Wash and dry DNA pellet	70% ethanol 2 ml 15,000 x g 5 min at RT 5-10 min	70% ethanol 2 ml / ≥ 3 x air	70% ethanol 5 ml 15,000 x g 10 min at RT 10-15 min	70% ethanol 5 ml / ≥ 3 x air
12 Reconstitute DNA	Appropriate volume of TE buffer	Buffer TRIS 500-1000 µl	Appropriate volume of TE buffer	Buffer TRIS 500-1000 µl

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1 Kit contents

	NucleoBond® Xtra Midi			NucleoBond® Xtra Midi Plus	
	10 preps	**50 preps**	**100 preps**	**10 preps**	**50 preps**
Cat. No.	**740410.10**	**740410.50**	**740410.100**	**740412.10**	**740412.50**
Buffer RES	100 ml	500 ml	1000 ml	100 ml	500 ml
Buffer LYS	4 x 25 ml	500 ml	1000 ml	4 x 25 ml	500 ml
Buffer NEU	100 ml	500 ml	1000 ml	100 ml	500 ml
Buffer EQU	200 ml	2 x 500 ml	2 x 1000 ml	200 ml	2 x 500 ml
Buffer WASH	100 ml	500 ml	1000 ml	100 ml	500 ml
Buffer ELU	60 ml	300 ml	600 ml	60 ml	300 ml
RNase A* (lyophilized)	6 mg	30 mg	2 x 30 mg	6 mg	30 mg
NucleoBond® Xtra Midi columns	10	50	100	10	50
NucleoBond® Xtra Midi column filters	10	50	100	10	50
NucleoBond® Finalizer	-	-	-	10	50
30 ml Syringes	-	-	-	10	50
1 ml Syringes	-	-	-	10	50
Buffer TRIS	-	-	-	15 ml	75 ml
Plastic washer	5	10	10	5	10
Protocol	1	1	1	1	1

* For preparation of working solutions and storage conditions see section 5.

1 Kit contents *continued*

	NucleoBond® Xtra Maxi			NucleoBond® Xtra Maxi Plus	
	10 preps	**50 preps**	**100 preps**	**10 preps**	**50 preps**
Cat. No.	**740414.10**	**740414.50**	**740414.100**	**740416.10**	**740416.50**
Buffer RES	150 ml	750 ml	2 x 750 ml	150 ml	750 ml
Buffer LYS	150 ml	750 ml	2 x 750 ml	150 ml	750 ml
Buffer NEU	150 ml	750 ml	2 x 750 ml	150 ml	750 ml
Buffer EQU	500 ml	2 x 1000 ml 500 ml	5 x 1000 ml	500 ml	2 x 1000 ml 500 ml
Buffer WASH	300 ml	1000 ml 500 ml	3 x 1000 ml	300 ml	1000 ml 500 ml
Buffer ELU	180 ml	900 ml	2 x 900 ml	180 ml	900 ml
RNase A* (lyophilized)	10 mg	50 mg	2 x 50 mg	10 mg	50 mg
NucleoBond® Xtra Maxi columns	10	50	100	10	50
NucleoBond® Xtra Maxi column filters	10	50	100	10	50
NucleoBond® Finalizer Large	-	-	-	10	50
30 ml Syringes	-	-	-	10	50
1 ml Syringes	-	-	-	10	50
Buffer TRIS	-	-	-	15 ml	75 ml
Plastic washer	5	10	10	5	10
Protocol	1	1	1	1	1

* For preparation of working solutions and storage conditions see section 5.

2 Kit specifications

- **NucleoBond® Xtra kits** are suitable for ultra fast purification of plasmids, cosmids, and very large constructs (P1 constructs, BACs, PACs) ranging from 3 kb up to 300 kb. For preparation of working solutions and storage conditions see section 5.

- **NucleoBond® Xtra columns** are polypropylene columns containing **NucleoBond® Xtra silica resin** packed between two inert filter elements. The columns are available in Midi and Maxi sizes with typical maximum DNA yields of 250 µg and 1000 µg, respectively.

- All **NucleoBond® Xtra columns** are resistant to organic solvents such as alcohol, chloroform, and phenol and are also suitable for buffers containing denaturing agents like formamide, urea, or common detergents like Triton X-100 or NP-40.

- **NucleoBond® Xtra silica resin** can be used over a wide pH range (pH 2.5–8.5), and can remain in contact with buffers for several hours without any change in its chromatographic properties.

- The **NucleoBond® Xtra column filters** are specially designed depth filters that fit into the NucleoBond Xtra columns. The filters are inserted ready-to-use in the **NucleoBond® Xtra columns** and allow a time-saving simultaneous clearing of bacterial lysate and loading of cleared lysate onto the NucleoBond Xtra column. Furthermore, the use of the column filters avoids the time-consuming centrifugation step for lysate clearing.

- The **NucleoBond® Xtra column filters** allow complete removal of precipitate even with large lysate volumes without clogging and avoid shearing of large DNA constructs, such as PACs or BACs by the gentle depth filter effect.

- The **NucleoBond® Xtra Midi Plus** and **NucleoBond® Xtra Maxi Plus** kits additionally contain the **NucleoBond® Finalizer** and **NucleoBond® Finalizer Large,** respectively. These tools for a fast concentration and desalination of eluates are suitable for most plasmids and cosmids ranging from 2-50 kb with recovery efficiencies from 40-90% (depending on elution volume).

- **NucleoBond® Finalizer** is a polypropylene syringe filter containing a special silica membrane. The **NucleoBond® Finalizer** provides a binding capacity of 500 µg, whereas the **NucleoBond® Finalizer Large** can hold up to 2000 µg plasmid DNA.

- Due to the small dead volumes of **NucleoBond® Finalizers** the plasmid DNA can be eluted with a concentration up to 2.0 µg/µl (see section 4.10, Figures 3 and 4 for dependence of concentration on elution volume).

- All **NucleoBond® Finalizers** are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of endotoxins.

3 About this user manual

The following section 4 provides you with a detailed description of the **NucleoBond® Xtra** purification system and important information about cell growth, cell lysis, and the subsequent purification steps. Sections 5 and 6 inform you about storage, buffer preparation, and safety instructions.

First-time users are strongly advised to read these chapters thoroughly before using this kit. Experienced users can directly proceed with the purification protocols (section 7) or just use the Protocol-at-a-glance for a quick reference.

Each procedural step in the purification protocol is arranged like the following example taken from section 7.1:

Midi	Maxi

5 Cell lysis

Check lysis buffer LYS for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30-40°C until precipitate is dissolved completely. Cool buffer down to room temperature (20-25°C).

Add **lysis buffer LYS** to the suspension.

Mix gently by **inverting** the tube **5 times**. **Do not vortex** as this will shear and release contaminating chromosomal DNA from cellular debris into the suspension.

Incubate the mixture at room temperature (20-25°C) for **5 min**.

Note: Increase LYS buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis).

8 ml	12 ml

If you are performing a Midi prep to purify plasmid DNA you will find volumes or incubation times in the white boxes. For Maxi preps please refer to the black boxes.

The name of the buffer, buffer volume, incubation times, repeats or important handling steps are emphasized in **bold type** within the instruction. Additional notes or optional steps are printed in italic. The exclamation point marks information and hints that are essential for a successful preparation.

In the example shown above you are asked to check the lysis buffer LYS prior to use and then to lyse the resuspended cell pellet in **8 ml** of **buffer LYS** when performing a Midi prep and in **12 ml** for a Maxi prep, respectively. Follow the handling instructions exactly and note the given hints for protocol alterations.

4 NucleoBond® Xtra plasmid purification system

4.1 Basic principle

The bacterial cells are lysed by an optimized set of newly formulated buffers based on the NaOH/SDS lysis method of Birnboim and Doly*.

After equilibration of the **NucleoBond® Xtra column** together with the corresponding **NucleoBond® Xtra column filter**, the entire lysate is loaded by gravity flow and simultaneously cleared by the newly designed column filter.

Plasmid DNA is bound to the improved **NucleoBond® Xtra silica resin**.

After an efficient washing step the plasmid DNA is eluted, precipitated, and easily dissolved in any suitable buffer (e.g. low-salt buffer or water) for further use.

4.2 NucleoBond® Xtra anion exchange columns

NucleoBond® Xtra is a silica-based anion-exchange resin, developed by MACHEREY-NAGEL and covered under European Patent EP 0496822. It is developed for routine separation of different classes of nucleic acids like oligonucleotides, RNA and plasmids.

NucleoBond® Xtra silica resin consists of hydrophilic, macroporous silica beads functionalized with MAE (methyl-amino-ethanol). The dense coating of this functional group provides a high overall positive charge density under acidic pH conditions that permits the negatively charged phosphate backbone of plasmid DNA to bind with high specificity (Figure 1).

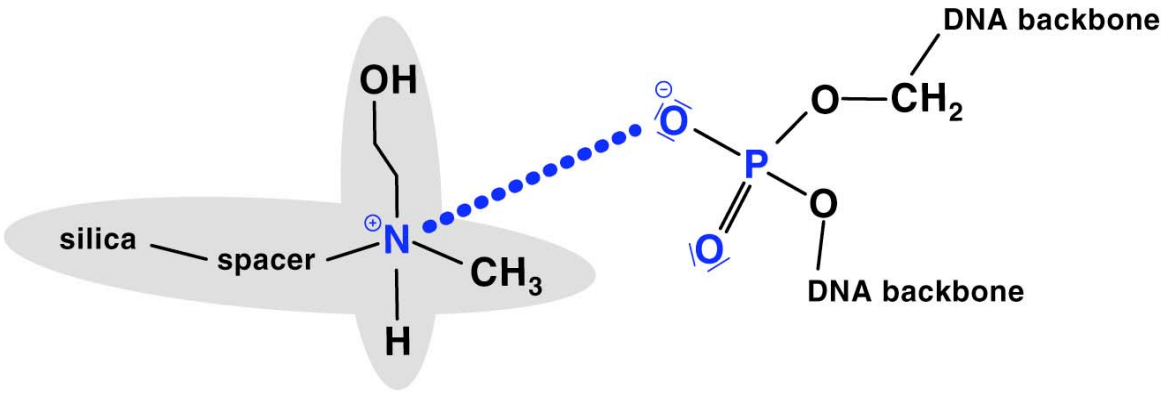


Figure 1 **Ionic interaction of the positively charged methyl-hydroxyethyl-amino group with the negative phosphate oxygen of the DNA backbone**. In contrast to the widely used DEAE (diethylaminoethanol) group, the hydroxy group of methyl-hydroxyethyl-amin can be involved in additional hydrogen bonding interactions with the DNA.

* Birnboim, H. C. and Doly, J., (1979) Nucl. Acids Res. 7, 1513-1523

Due to a specialized manufacturing process that is strictly controlled and monitored, the **NucleoBond® Xtra** silica beads are uniform in diameter and contain particularly large pores. These special properties allow optimized flow rates and sharp, well-defined elution profiles. **NucleoBond® Xtra** can separate distinct nucleic acid species from each other and from proteins, carbohydrates, and other unwanted cellular components over an exceptionally broad range of salt concentrations (Figure 2).

All contaminants from proteins to RNA are washed from the column, the positive charge of the resin is neutralized by a pH shift to slightly alkaline conditions and pure plasmid DNA is eluted in a high-salt elution buffer.

The purified nucleic acid products are suitable for use in the most demanding molecular biology applications, including transfection, *in vitro* transcription, automated or manual sequencing, cloning, hybridization, and PCR.

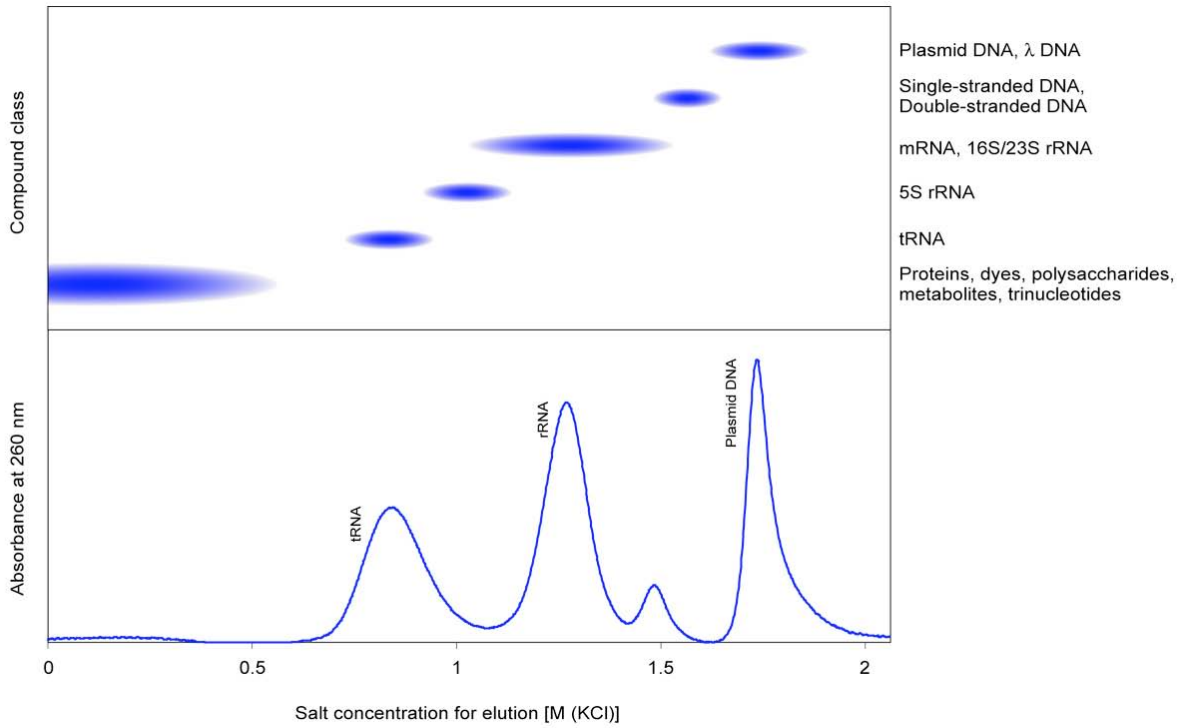


Figure 2 Elution profile of NucleoBond® Xtra silica resin at pH 7.0
The more interactions a nucleic acid can form between phosphate backbone and the positively charged resin the later it is eluted with increasing salt concentration. Large nucleic acids carry more charges than short ones, double stranded DNA more than single stranded RNA.

4.3 Growth of bacterial cultures

Yield and quality of plasmid DNA highly depend on the **type of culture media** and antibiotics, the bacterial host strain, the plasmid type, size, and copy number.

For standard high-copy plasmids LB (Luria-Bertani) medium is recommended. The cell culture should be incubated at 37°C with constant shaking (200-250 rpm) preferably 12-16 h over night. Use flasks of at least three or four times the volume of the culture volume to provide a growth medium saturated with oxygen. Alternatively, rich media like 2xYT (Yeast/Tryptone), TB (Terrific Broth) or CircleGrow can be used. In this case bacteria grow faster, reach the stationary phase much earlier than in LB medium (≤ 12 h), and higher cell masses can be reached. However, this does not necessarily yield more plasmid DNA. Overgrowing a culture might lead to a higher percentage of dead or starving cells and the resulting plasmid DNA might be partially degraded or contaminated with chromosomal DNA. To find the optimal culture conditions, the culture medium and incubation times have to be optimized for each host strain/plasmid construct combination individually.

Cell cultures should be grown under **antibiotic selection** at all times to ensure plasmid propagation. Without this selective pressure, cells tend to lose a plasmid during cell division. Since bacteria grow much faster without the burden of a high-copy plasmid, they take over the culture rapidly and the plasmid yield goes down regardless of the cell mass. Table 1 gives information on concentrations of commonly used antibiotics.

Table 1: Information about antibiotics according to Maniatis*

Antibiotic	**Stock solution (concentration)**	**Storage**	**Working concentration**
Ampicillin	50 mg/ml in H_2O	-20°C	50-100 µg/ml
Chloramphenicol	34 mg/ml in EtOH	-20°C	25-170 µg/ml
Kanamycin	10 mg/ml in H_2O	-20°C	10-50 µg/ml
Streptomycin	10 mg/ml in H_2O	-20°C	10-50 µg/ml
Tetracycline	5 mg/ml in EtOH	-20°C	10-50 µg/ml

The ***E. coli* host strain** mostly influences the quality of the plasmid DNA. Whereas strains like DH5a or XL1-Blue usually produce high quality super-coiled plasmid DNA, other strains like e.g. HB101 with high levels of endonuclease activity might yield lower quality plasmid giving poor results in downstream applications like enzymatic restriction or sequencing.

* Maniatis T, Fritsch EF, Sambrook J: *Molecular cloning. A laboratory manual,* Cold Spring Harbor, Cold Spring, New York 1982.

The **type of plasmid**, especially the **size and the origin of replication (ori)** has a crucial influence on DNA yield. In general, the larger the plasmid or the cloned insert is, the lower is the expected DNA yield due to a lower copy number. Even a high-copy construct based on a ColE1 ori can behave like a low-copy vector in case of a large or unfavorable insert. In addition, the ori itself influences the yield by factor 10 - 100. Thus plasmids based on e.g. pBR322 or pACYC, cosmids or BACs are maintained at copy numbers <20 down to even only 1, whereas vectors based on e.g. pUC, pBluescript or pGEM can be present in several hundred copies per cell.

Therefore, all the above mentioned factors should be taken into consideration if a particular DNA yield has to be achieved. Culture volume and lysis procedures have to be adjusted accordingly.

4.4 Culture volume for high-copy plasmids

Due to the influence of growth media (TB, CircleGrow, 2xYT), growth conditions (shaking, temperature), host strain or type of plasmid insert etc. the final amount of cells in a bacterial culture can vary over a wide range. E. g. overnight cultures in flasks usually reach an OD_{600} of 3-6 under vigorous shaking, while fermentation cultures reach an OD_{600} of 10 and more. By rule of thumb, 1 liter of *E. coli* culture grown in LB medium yields a pellet of about 5-10 g wet weight. The expected DNA yield for a high-copy plasmid is 1 mg per gram cell wet weight.

It is therefore important to adjust the cell mass rather than the culture volume for the best plasmid purification results. But since the cell mass or cell wet weight is tedious to determine it was replaced in this manual by the mathematical product of optical density at 600 nm (OD) and culture volume (V) - two variables that are much easier to measure.

$$\mathbf{ODV = OD_{600} \times Vol\ [ml]}$$

Table 2: Recommended culture volumes for high-copy plasmids

NucleoBond® Xtra Kit	Pellet wet weight	Rec. ODV	Recommended culture volume for				
			OD_{600} = 2	OD_{600} = 4	OD_{600} = 6	OD_{600} = 8	OD_{600} = 10
Midi	0.75 g	400	200 ml	100 ml	66 ml	50 ml	40 ml
Maxi	2.25 g	1200	600 ml	300 ml	200 ml	150 ml	120 ml

Table 2 shows recommended ODVs and the corresponding pairs of OD_{600} and culture volume that can be easily handled using the standard kit protocol lysis buffer volumes.

4.5 Culture volume for low-copy plasmids

NucleoBond® Xtra kits are designed for isolation of high-copy plasmids (up to several hundred copies/cell) as well as low-copy plasmids (<20 copies/cell). However, when purifying low-copy plasmids, the cell mass and the lysis buffer volumes should be increased at least by factor 2 to provide enough DNA to utilize the columns´ binding capacity. Table 3 shows recommended ODVs and the corresponding pairs of OD_{600} and culture volume for low-copy plasmid cell cultures (for detailed information on calculating ODV = OD_{600} x Vol refer to section 4.4).

For higher yields, it is advantageous to increase the cell culture and lysis buffer volumes even more, e. g. by factor 3-5. In this case additional lysis buffer can be ordered separately (see section 9.2 for ordering information). Furthermore, a centrifuge should be used for lysate clarification instead of the provided **NucleoBond® Xtra column filters** since their capacity for precipitate is limited.

Table 3: Suggested culture volumes for low-copy plasmids

NucleoBond® Xtra Kit	Pellet wet weight	Rec. ODV	Recommended culture volume for				
			OD_{600} = 2	OD_{600} = 4	OD_{600} = 6	OD_{600} = 8	OD_{600} = 10
Midi	1.50 g	800	400 ml	200 ml	133 ml	100 ml	80 ml
Maxi	4.50 g	2400	1200 ml	600 ml	400 ml	300 ml	240 ml

4.6 Cell lysis

The bacterial cell pellet is resuspended in buffer RES and lysed by a sodium hydroxide/SDS treatment with buffer LYS. Proteins, as well as chromosomal and plasmid DNA are denatured under these conditions. RNA is degraded by DNase-free RNase A. Neutralization buffer NEU, containing potassium acetate, is then added to the lysate, causing SDS to precipitate as KDS (potassium dodecyl sulfate) and pulling down proteins, chromosomal DNA, and other cellular debris. The potassium acetate buffer also neutralizes the lysate. Plasmid DNA can revert to its native super-coiled structure and remains in solution.

The **NucleoBond® Xtra** buffer volumes (standard protocol) are adjusted to ensure optimal lysis for culture volumes, appropriate for high-copy plasmids according to Table 2. Using too much cell material leads to inefficient cell lysis and precipitation and might reduce your plasmid yield and purity. Therefore, lysis buffer volumes should be increased when applying larger culture volumes in case of e. g. low-copy vector purification (section 4.5, Table 3).

By rule of thumb, calculate the necessary lysis buffer volumes for RES, LYS and NEU as follows:

Vol [ml] = Culture Volume [ml] x OD_{600} / 50

For example, if 200 ml of a low-copy bacterial culture (OD_{600} = 4) is to be lysed, the appropriate volumes of lysis buffers RES, LYS, and NEU are 16 ml each. If more lysis buffer is needed than is provided with the kit, an additional buffer set including buffers RES, LYS, NEU, and RNase A can be ordered separately. Please refer to section 9.2 for ordering information.

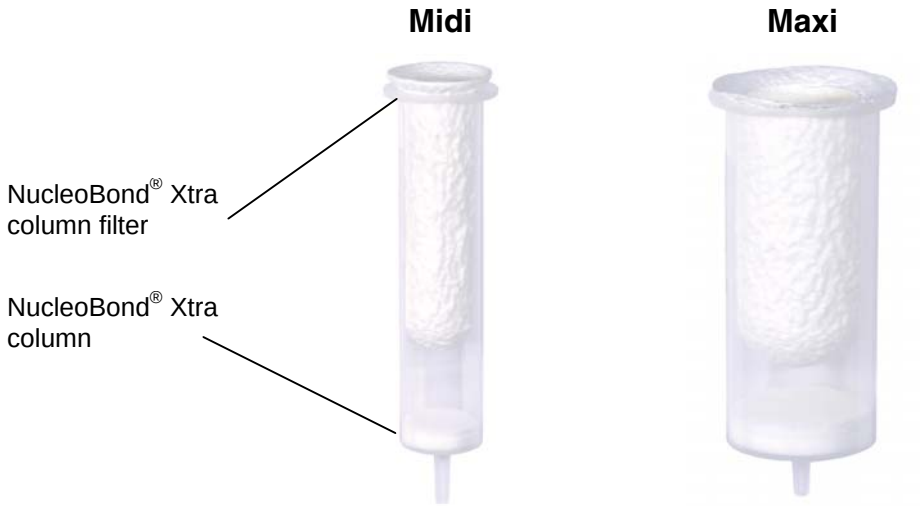
By using sufficient amounts of lysis buffer, lysis time can be limited to 3-4 minutes and should not exceed 5 minutes. Prolonged exposure to alkaline conditions can irreversibly denature and degrade plasmid DNA and liberate contaminating chromosomal DNA into the lysate.

4.7 Difficult-to-lyse strains

For plasmid purification of e.g. Gram positive bacteria or strains with a more resistant cell wall it might be advantageous to start the preparation with a lysozyme treatment. Therefore, resuspend the cell pellet in buffer RES containing **2 mg/ml lysozyme** and incubate at **37°C** for **30 minutes**. Proceed then with the lysis procedure according to the **NucleoBond® Xtra** standard protocol.

4.8 Filtration and loading of the lysate

After the alkaline lysis, the sample has to be cleared from cell debris and precipitate to ensure high plasmid purity and a fast column flow rate. This is achieved by passing the solution through a **NucleoBond® Xtra column filter** which is provided already inserted into the **NucleoBond® Xtra column**.



The **NucleoBond® Xtra column filters** are designed to eliminate the centrifugation step after alkaline lysis. They are pre-wet during column equilibration and allow a time-saving simultaneous clearing of bacterial lysate and loading of the **NucleoBond® Xtra column**.

Compared to lysate clearing by centrifugation or syringe filters the **NucleoBond® Xtra column filter** furthermore avoids shearing of large DNA constructs such as PACs or BACs by the gentle depth filter effect (filtration occurs on the surface of the filter as well as inside the filter matrix). Its special material and design lead to very rapid passage of the lysate through the filter and even very large lysate volumes can be applied without the risk of clogging. This is especially important for e. g. low-copy plasmid purification. However, if more than the recommended cell mass (see section 4.4, Table 2, section 4.5, Table 3) was lysed, it might be advantageous to use a centrifuge for lysate clarification rather than the provided column filters due to their limited precipitate capacity.

4.9 Washing of the column

The high salt concentration of the lysate prevents proteins and RNA from binding to the **NucleoBond® Xtra column** (see section 4.2, Figure 2). However, to remove all traces of contaminants and to purge the dead volume of the **NucleoBond® Xtra column filters** it is important to wash the column and the filter in two subsequent washing steps.

First apply buffer EQU to the funnel rim of the filter to wash all residual lysate out of the filter onto the column. Do not just pour the buffer inside the filter. Then pull out and discard the column filter or remove the filter by turning the column upside down. It is essential to wash the **NucleoBond® Xtra column** without filter for a second time with wash buffer WASH. This ensures highest yields with best achievable purity.

4.10 Elution and concentration of plasmid DNA

Elution is carried out under high-salt conditions and by a shift of pH from 6.5 to 9.0. Under these alkaline conditions the positive charge of the anion exchange resin is neutralized and plasmid DNA is released. For any downstream applications it is necessary to precipitate the DNA and to remove salt and all traces of alcohol since they disturb or inhibit enzymatic activity needed for restriction or sequencing reactions.

All **NucleoBond® Xtra** eluates already contain enough salt for an isopropanol precipitation of DNA. Therefore the precipitation is started by directly adding 0.7 volumes of isopropanol. To prevent co-precipitation of salt, use **room-temperature (20-25°C) isopropanol** only and do not let the plasmid DNA solution drop into a vial with isopropanol but **add isopropanol to the final eluate and mix immediately**.

Afterwards either follow the centrifugation protocol given after the **NucleoBond® Xtra** purification protocol or follow the support protocol for the **NucleoBond® Finalizers** in section 8 to eliminate the time-consuming centrifugation steps for precipitation (use of **NucleoBond® Finalizers** is only recommended for vector sizes smaller than 50 kb).

The **NucleoBond® Finalizers** are designed for quick concentration and desalination of plasmid and cosmid DNA eluates that are obtained by anion-exchange chromatography based DNA purification. The sample is precipitated with isopropanol as mentioned above and loaded onto a special silica membrane by means of a syringe. After an ethanolic washing step the membrane is dried by pressing air through the filter. Elution of pure DNA is carried out with slightly alkaline low salt buffers like **buffer TRIS** (5 mM Tris/HCl, pH 8.5, provided with the NucleoBond® Xtra Plus kits) or TE buffer (10 mM Tris/HCl, pH7.5, 1 mM EDTA).

For maximum yield it is recommended to perform the elution step twice. The first elution step is done using fresh buffer whereas in the second elution step the eluate from the first elution is reapplied on the **NucleoBond® Finalizer** to allow complete solubilization of the plasmid.

DNA recovery highly depends on the used elution buffer volume. Large volumes result in a high recovery of up to 90% but a lower DNA concentration. Small elution volumes on the other hand increase the concentration but at the cost of DNA yield.

Refer to Figure 3 and Figure 4 to select an elution buffer volume that meets your needs best.

If a small volume is chosen, make sure to recover as much eluate as possible from the syringe and **NucleoBond® Finalizer** by pressing air through the **NucleoBond® Finalizer** several times after elution and collecting every single droplet to minimize the dead volume.

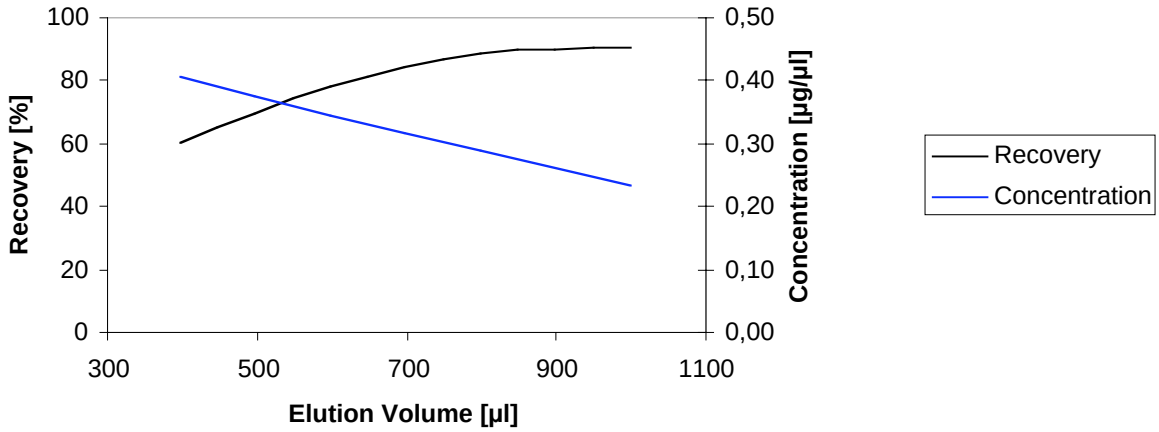


Figure 3 Concentration of NucleoBond® Xtra Midi eluates using NucleoBond® Finalizer
Final recovery and concentration of 250 µg plasmid DNA (8 kbp) loaded onto a NucleoBond® Finalizer and eluted two-fold with varying volumes of low salt buffer.

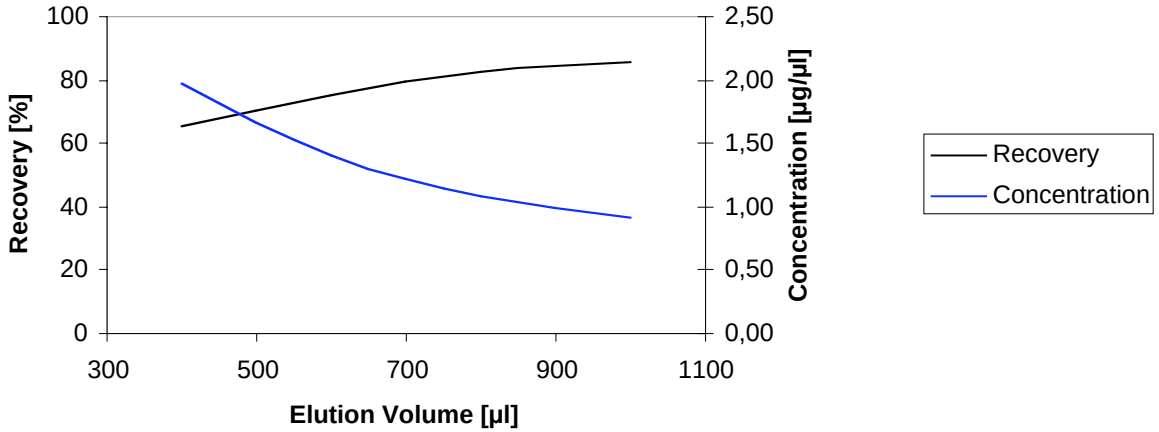


Figure 4 Concentration of NucleoBond® Xtra Maxi eluates using NucleoBond® Finalizer Large
Final recovery and concentration of 1000 µg plasmid DNA (8 kbp) loaded onto a NucleoBond® Finalizer and eluted two-fold with varying volumes of low salt buffer.

NucleoBond® Finalizer is designed to hold a maximum of 500 µg DNA and is therefore ideally suited for the use in combination with a **NucleoBond® Xtra Midi** kit. **NucleoBond® Xtra Maxi** eluates are easily concentrated with a **NucleoBond® Finalizer Large** which is able to bind up to 2000 µg plasmid DNA. Optimal recoveries are achieved with both **NucleoBond® Finalizers** by using 1000 µl of elution buffer.

4.11 Determination of DNA yield and quality

The **yield** of a plasmid preparation should be estimated prior to and after the isopropanol precipitation in order to calculate the recovery after precipitation and to find the best volume to dissolve the pellet in. Just use either **NucleoBond® Xtra** elution buffer ELU or the respective low-salt buffer as a blank in your photometric measurement.

The nucleic acid **concentration** of the sample can be calculated from its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µg DNA/ml. Note that the absolute measured absorbance should lay between 0.1 and 0.7 to be in the linear part of Lambert-Beer´s law. Dilute your sample in the respective buffer if necessary.

The plasmid **purity** can be checked by UV spectroscopy as well. A ratio of $A_{260/280}$ between 1.80-1.90 and $A_{260/230}$ around 2.0 indicates pure plasmid DNA. An $A_{260/280}$ ratio above 2.0 is a sign for too much RNA in your preparation, an $A_{260/280}$ ratio below 1.8 indicates protein contamination.

Plasmid **quality** can be checked by running the precipitated samples on a 1% agarose gel. This will give information on conformation and structural integrity of isolated plasmid DNA i.e. it shows whether the sample is predominantly in the favorable super-coiled (ccc, usually the fastest band), an open circle (oc) or even linear form (see section 9.1, Figure 3).

4.12 Convenient stopping points

Cell pellets can easily be stored for several months at -20°C.

Cleared lysates can be kept on ice or at 4°C for several days.

Once the column purification is started it should not be interrupted for more than an hour. The columns can be left unattended for several minutes since they do not run dry but complete drying out due to evaporation should be avoided at any rate. You should proceed to the elution step after which the eluate can be stored for several days at 4°C. Note that the eluate should be warmed up to room temperature before precipitating the DNA to avoid co-precipitation of salt.

5 Storage conditions and preparation of working solutions

All kit components can be stored at room temperature (20-25°C) and are stable up to two years.

Before you start any **NucleoBond® Xtra** plasmid purification prepare the following:

- Dissolve the lyophilized RNase A* by the addition of 1 ml of buffer RES. Wearing gloves is recommended. Pipette up and down until the RNase A is dissolved completely. Transfer the RNase A solution back to the bottle containing buffer RES and shake well. Note the date of RNase A addition. The final concentration of RNase A is 60 µg/ml buffer RES. Store buffer RES with RNase A at 4°C. The solution will be stable at this temperature at least up to 6 months.

- Buffer LYS should be stored at room temperature (20-25°C) since the containing SDS might precipitate at temperatures below 20°C. If precipitation is observed, incubate the bottle for several minutes at about 30-40°C and mix well until the precipitate is redissolved.

* Ref. 740410.100 contains **2 x 30 mg of RNase A**. Make sure to dissolve RNase A of both vials, each in 1 ml of buffer RES, and transfer the solution back into the bottle containing buffer RES.

6 Safety instructions - risk and safety phrases

The following components of the **NucleoBond® Xtra** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
RNase A	RNase A, lyophilized		Xi* May cause sensitization by inhalation and skin contact	R 42/43	S 22-24
LYS	sodium hydroxide < 2 %		Xi** Irritating to eyes and skin	R 36/38	S 26-37/39 S 45

Risk Phrases

R 36/38 Irritating to eyes and skin

R 42/43 May cause sensitization by inhalation and skin contact

Safety Phrases

S 22 Do not breathe dust

S 24 Avoid contact with the skin

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S 37/39 Wear suitable gloves and eye/face protection

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

** Label not necessary, if quantity below 25 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

7 NucleoBond® Xtra plasmid purification

The following section includes the protocols for high-copy and low-copy plasmid purification in English, German, and French.

7.1 High-copy plasmid purification (Midi, Maxi) - English

Midi	Maxi

1 Prepare a starter culture

Inoculate a 3-5 ml starter culture of LB medium with a single colony picked from a freshly streaked agar plate. Make sure that plate and liquid culture contain the appropriate selective antibiotic to guarantee plasmid propagation (see section 4.3 for more information). Shake at 37°C and ~300 rpm for ~8 h.

2 Prepare a large overnight culture

Inoculate an overnight culture by diluting the starter culture 1/1000 into the given volumes of LB medium also containing the appropriate selective antibiotic. If the culture is known to grow poorly or the plasmid does not quite behave like a high-copy plasmid, please consult section 4.4 for larger culture volumes.

Grow the culture overnight at 37°C and ~300 rpm for 12–16 h.

Note: To utilize the entire large binding capacity of the NucleoBond® Xtra columns it is important to provide enough plasmid DNA. If you are not sure about the plasmid copy number and growth behavior of your host strain, increase the culture volume and decide in step 3 how much cells to use for the preparation.

100 ml	300 ml

3 Harvest bacterial cells

Measure the cell culture OD_{600} and determine the recommended culture volume

$V\ [ml] = 400 / OD_{600}$	$V\ [ml] = 1200 / OD_{600}$

Pellet the cells by centrifugation at **4,500 - 6,000 x g** for **≥10 min** at **4°C** and discard the supernatant completely.

Note: It is of course possible to use larger culture volumes, e. g. if the plasmid does not behave like a typical high-copy vector (see section 4.4 for more information). In this case increase RES, LYS and NEU buffer volumes proportionally in steps 4, 5 and 7. If the culture volume is more than double the recommended culture volume, it is advantageous to use a centrifuge for the lysate clarification in step 8 rather than the NucleoBond® Xtra column filters.

Midi	Maxi

4 Resuspension

Resuspend the cell pellet completely in **resuspension buffer RES + RNase A** by pipetting the cells up and down.

For an efficient cell lysis it is important that no clumps remain in the suspension.

Note: Increase RES buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis and section 4.7 regarding difficult-to-lyse strains).

8 ml	12 ml

5 Cell lysis

Check lysis buffer LYS for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30-40°C until precipitate is dissolved completely. Cool buffer down to room temperature (20-25°C).

Add **lysis buffer LYS** to the suspension.

Mix gently by **inverting** the tube **5 times**. **Do not vortex** as this will shear and release contaminating chromosomal DNA from cellular debris into the suspension.

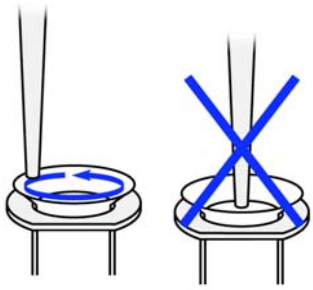
Incubate the mixture at room temperature (20-25°C) for **5 min**.

Note: Increase LYS buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis).

8 ml	12 ml

6 Equilibration

Equilibrate a NucleoBond® Xtra column together with the inserted column filter with **equilibration buffer EQU**.



Apply the buffer onto the rim of the column filter as shown in the picture. Allow the column to empty by gravity flow and make sure to wet the entire filter.

The column does not run dry.

12 ml	25 ml

Midi	Maxi

7 Neutralization

Add **neutralization buffer NEU** to the suspension and immediately mix the lysate gently by **inverting** the tube **10-15 times**. **Do not vortex.**

The flask or tube used for this step should not be filled more than two thirds to allow homogeneous mixing. Make sure to neutralize completely to precipitate all the protein and chromosomal DNA. The lysate should turn from a slimy, viscous consistency to a low viscosity, homogeneous suspension of an off-white flocculate.

Immediately proceed with step 8. **An incubation of the lysate is not necessary.**

Note: Increase NEU buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis).

8 ml	12 ml

8 Clarification and loading

Make sure to have a homogeneous suspension of the precipitate by **inverting the tube 3 times** directly before applying the lysate to the equilibrated NucleoBond® Xtra column filter to avoid clogging of the filter.

The lysate is simultaneously cleared and loaded onto the column. Refill the filter if more lysate has to be loaded than the filter is able to hold. Allow the column to empty by gravity flow.

Alternative: The precipitate can be removed by centrifugation at ≥5,000 x g for at least 10 min, e.g. if more than double the recommended cell mass was used. If the supernatant still contains suspended matter transfer it to a new tube and repeat the centrifugation, preferably at higher speed, or apply the lysate to the equilibrated NucleoBond® Xtra column filter.

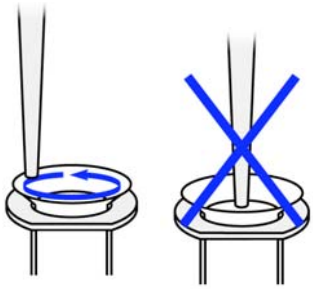
This clarification step is extremely important since residual precipitate may clog the NucleoBond® Xtra column. To load the column you can either apply the cleared lysate to the equilibrated filter or remove the unused filter beforehand. Allow the column to empty by gravity flow.

Note: You may want to save all or part of the flow-through for analysis (see section 9.1).

Midi	Maxi

9 Wash column filter and column (equilibration buffer EQU)

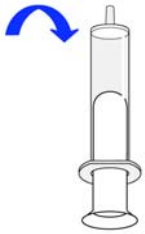
Wash the NucleoBond® Xtra column filter and NucleoBond® Xtra column with **equilibration buffer EQU**.



Apply the buffer to the funnel shaped rim of the filter and make sure it is washing out the lysate which is remaining in the filter. Omitting this step or just pouring the buffer directly inside the funnel may reduce plasmid yield.

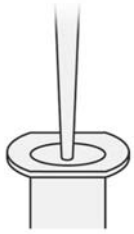
5 ml	15 ml

10 Discard column filter



Either pull out the NucleoBond® Xtra column filter or discard it by turning the column upside down.

11 Wash column (washing buffer WASH)



Wash the NucleoBond® Xtra column with **washing buffer WASH**. It is important to remove the column filter before applying the washing buffer to avoid low purity.

8 ml	25 ml

12 Elution

Elute the plasmid DNA with **elution buffer ELU**.

Proceed with **step 13** for the centrifugation protocol after isopropanol precipitation or continue with **section 8** for plasmid concentration and desalination by using the NucleoBond® Finalizer (NucleoBond® Xtra Midi Plus) or NucleoBond® Finalizer Large (NucleoBond® Xtra Maxi Plus).

Optional: Determine plasmid yield by UV spectrophotometry in order to adjust desired concentration of DNA in step 15 and calculate the recovery after precipitation.

5 ml	15 ml

Midi	Maxi

13 Precipitation

Add **room-temperature isopropanol** to precipitate the eluted plasmid DNA. Vortex well and let the mixture sit for **2 minutes**.

Centrifuge at **≥ 5,000 x g** for **≥15 min** at **≤ room temperature,** preferably at **15,000 x g** for **30 min** at **4°C**. Carefully discard the supernatant.

3.5 ml	10.5 ml

14 Wash and dry DNA pellet

Add **room-temperature 70% ethanol** to the pellet and centrifuge at **≥ 5,000 x g**, preferably **≥ 15,000 x g** for **5 min** at **room temperature** (20-25°C).

2 ml	5 ml

Carefully remove ethanol completely from the tube with a pipette tip. Allow the pellet to dry at **room temperature** (20-25°C).

Note: Plasmid DNA might be harder to dissolve when over-dried.

5-10 min	10-15 min

15 Reconstitute DNA

Dissolve the DNA pellet in an appropriate volume of buffer TE or sterile deionized H_2O. Depending on the type of centrifugation tube, dissolve under gentle pipetting up and down or constant spinning in a sufficient amount of buffer for 10-60 min (3D-shaker).

Determine plasmid yield by UV spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis (see section 4.11).

7.2 High-copy plasmid purification (Midi, Maxi) - German

Midi | **Maxi**

1 Ansetzen einer Vorkultur

Beimpfen Sie 3-5 ml LB Medium mit einer einzelnen Kolonie einer frisch ausgestrichenen Agarplatte. Stellen Sie sicher, dass sowohl die Platte als auch das Flüssigmedium das nötige Antibiotikum enthält, da bei fehlendem Selektionsdruck die Bakterien ihr Plasmid bei der Zellteilung verlieren können (für weitere Informationen siehe Kapitel 4.3). Schütteln Sie die Vorkultur bei 37°C und ~300 rpm für ~8 h.

2 Ansetzen einer Übernachtkultur

Beimpfen Sie LB Medium des unten angegebenen Volumens durch Verdünnen der Vorkultur um den Faktor 1/1000. Stellen Sie sicher, dass das Medium das nötige Antibiotikum enthält. Wählen Sie ein größeres Volumen (Kapitel 4.4), falls die Kultur bekanntermaßen langsam oder schlecht wächst oder das Plasmid sich nicht wie ein high-copy Plasmid verhält.

Inkubieren Sie auf einem Schüttler bei 37°C und ~300 rpm für ~12–16 h.

Hinweis: Um die hohe Bindekapazität der NucleoBond® Xtra Säulen voll ausnutzen zu können ist es wichtig, ausreichend Plasmid DNA zu laden. Sollten Sie bzgl. Kopienzahl des Plasmids und Wachstumsverhaltens des Bakterienstamms unsicher sein, erhöhen Sie das Kulturvolumen und entscheiden in Schritt 3 wie viele Zellen für die Präparation eingesetzt werden.

100 ml | **300 ml**

3 Ernte der Bakterienzellen

Messen Sie die OD_{600} der Bakterienkultur und bestimmen Sie das empfohlene **Kulturvolumen** gemäß folgender Formel

V [ml] = 400 / OD_{600} | **V [ml] = 1200 / OD_{600}**

Pelletieren Sie die Zellen durch Zentrifugation bei **4,500 - 6,000 x g** für **≥10 min** bei **4°C** und entfernen Sie den Überstand vollständig.

Hinweis: Es können auch größere Kulturvolumina verwendet werden, z.B. wenn sich das Plasmid nicht wie ein typischer high-copy Vektor verhält (für weitere Informationen siehe Kapitel 4.4). In diesem Fall erhöhen Sie die Volumina der Puffer RES, LYS und NEU proportional in den Schritten 4, 5 und 7. Falls das Kulturvolumen mehr als doppelt so hoch ist wie das empfohlene, verwenden Sie in Schritt 8 zur Lysatklärung eine Zentrifuge anstelle des NucleoBond® Xtra column filters.

Midi	Maxi

4 Resuspension

Resuspendieren Sie das Zellpellet vollständig in **resuspension buffer RES + RNase A** durch Auf- und Abpipettieren.

Wichtig für eine effiziente Zelllyse ist, dass keine Zellklumpen in der Suspension verbleiben.

Hinweis: Erhöhen Sie das Volumen des Puffers RES proportional falls mehr als die empfohlene Zellmasse eingesetzt wird (für Informationen zur optimalen Zelllyse siehe Kapitel 4.6, für schwer zu lysierende Bakterienstämme siehe Kapitel 4.7).

8 ml	12 ml

5 Zelllyse

Vor Gebrauch überprüfen Sie lysis buffer LYS auf ausgefallenes SDS. Sollte ein weißes Präzipitat sichtbar sein, erwärmen Sie den Puffer für einige Minuten auf 30-40°C bis das Präzipitat komplett gelöst ist. Lassen Sie den Puffer auf Raumtemperatur (20-25°C) abkühlen.

Geben Sie **lysis buffer LYS** zu der Suspension.

Mischen Sie vorsichtig durch **5-maliges Invertieren** des Gefäßes. **Vortexen Sie nicht,** da dies zur Scherung der genomischen DNA und zu deren Freisetzung aus den Zelltrümmern in die Suspension führen kann.

Inkubieren Sie die Mischung für **5 min** bei Raumtemperatur (20-25°C).

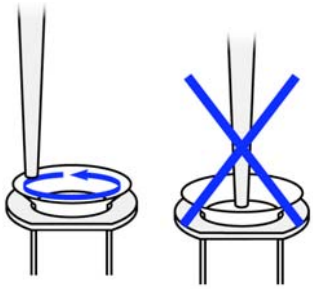
Hinweis: Erhöhen Sie das Volumen des Puffers LYS proportional falls mehr als die empfohlene Zellmasse eingesetzt wird (für Informationen zur optimalen Zelllyse siehe Kapitel 4.6).

8 ml	12 ml

Midi	Maxi

6 Äquilibrierung

Äquilibrieren Sie eine NucleoBond® Xtra Säule zusammen mit dem eingesetzten NucleoBond® Xtra Filter mit **equilibration buffer EQU**.



Geben Sie den Puffer auf den äußeren Rand des Filters wie in der Abbildung rechts gezeigt. Lassen Sie die Flüssigkeit vollständig durch die Säule laufen und stellen Sie sicher, daß der NucleoBond® Xtra Filter komplett benetzt ist.

Die Säulen laufen nicht trocken.

12 ml	25 ml

7 Neutralisation

Geben Sie **neutralization buffer NEU** zu der Suspension und mischen Sie sofort aber vorsichtig durch **10- bis 15-maliges Invertieren**. **Vortexen Sie nicht.**

Das für diesen Schritt verwendete Gefäß sollte nicht mehr als zwei Drittel gefüllt sein, um ein gleichmäßiges Durchmischen zu ermöglichen. Stellen Sie sicher, dass die Neutralisation vollständig ist, um eine quantitative Fällung von Protein und genomischer DNA zu gewährleisten. Das schleimige, viskose Lysat sollte nach Zugabe von **buffer NEU** dünnflüssig werden und eine homogene Suspension mit flockigem, weißem Präzipitat ausbilden.

Fahren Sie sofort mit Schritt 8 fort. **Eine Inkubation des Lysates ist nicht notwendig.**

Hinweis: Hinweis: Erhöhen Sie das Volumen des Puffers NEU proportional falls mehr als die empfohlene Zellmasse eingesetzt wird (für Informationen zur optimalen Zelllyse siehe Kapitel 4.6).

8 ml	12 ml

Midi	Maxi

8 Klärung des Lysates und Beladung der Säule

Um eine gleichmäßige Suspension des Präzipitates zu erzielen und somit ein Verstopfen des NucleoBond® Xtra Filters zu vermeiden, **invertieren** Sie das Gefäß erneut **3-malig** unmittelbar bevor Sie das Lysat auf den äquilibrierten Filter geben.

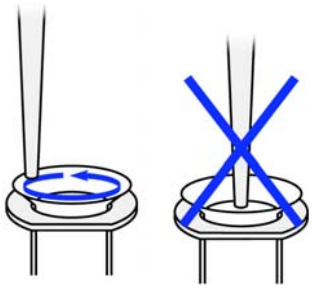
Das Lysat wird gleichzeitig geklärt und auf die NucleoBond® Xtra Säule geladen. Füllen Sie den Filter nach, falls mehr Lysat geladen werden soll, als der Filter auf einmal fassen kann. Lassen Sie die Flüssigkeit vollständig durch die Säule laufen.

Alternativ: Das Präzipitat kann alternativ mittels Zentrifugation bei ≥5,000 x g für mindestens 10 Min entfernt werden, wenn z.B. mehr als das Doppelte der empfohlenen Zellmasse verwendet wurde. Sollte der Überstand noch nicht völlig geklärt sein, überführen Sie ihn in ein neues Gefäß und wiederholen Sie die Zentrifugation, vorzugsweise bei höherer Geschwindigkeit oder geben Sie ihn auf den NucleoBond® Xtra Filter.

Diese Lysatklärung ist sehr wichtig, da restliches Präzipitat die NucleoBond® Xtra Säule verstopfen könnte. Um die Säule zu beladen, kann das geklärte Lysate entweder auf den äquilibrierten Filter oder nach Entfernung des unbenutzten Filters direkt auf die äquilibrierte Säule geladen werden. Lassen Sie die Flüssigkeit vollständig durch die Säule laufen.

Hinweis: An diesem Punkt der Prozedur kann ein Teil bzw. der gesamte Durchfluss der Säule für analytische Zwecke aufgehoben werden (siehe Kapitel 9.1).

9 Waschen des Filters und der Säule (equilibration buffer EQU)



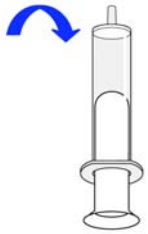
Waschen Sie den NucleoBond® Xtra Filter und die NucleoBond® Xtra Säule mit **equilibration buffer EQU**.

Geben Sie den Puffer auf den äußeren Rand des Säulenfilters wie in der Abbildung rechts gezeigt und stellen Sie sicher, dass das im Filter verbliebene Lysat ausgewaschen wird. Wird dieser Schritt weggelassen oder der Puffer direkt in den Säulenfilter statt auf den Rand gegeben, kann dies zu reduzierter Plasmid-ausbeute führen.

5 ml	15 ml

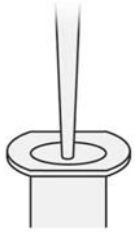
Midi	Maxi

10 Entfernen des Filters



Entnehmen Sie den NucleoBond® Xtra Filter oder entfernen Sie ihn durch einfaches Umdrehen der Säule.

11 Waschen der Säule (washing buffer WASH)



Waschen Sie die NucleoBond® Xtra Säule mit **washing buffer WASH**. Es ist wichtig, den Filter vor Aufgabe des Waschpuffers zu entfernen, da sonst die Reinheit der DNA negativ beeinflusst werden kann.

8 ml	25 ml

12 Elution

Eluieren Sie die Plasmid DNA mit **elution buffer ELU**.

Fahren Sie mit **Schritt 13** fort, um die Isopropanol-Fällung gemäß Zentrifugationsprotokoll durchzuführen oder folgen Sie der Anleitung in **Kapitel 8** zur Konzentrierung und Entsalzung mittels NucleoBond® Finalizer (NucleoBond® Xtra Midi Plus) oder NucleoBond® Finalizer Large (NucleoBond® Xtra Maxi Plus).

Optional: Bestimmen Sie photometrisch die Plasmidausbeute, um in Schritt 15 die gewünschte DNA-Konzentration einstellen und die endgültige Ausbeute nach der Präzipitation bestimmen zu können.

5 ml	15 ml

13 Präzipitation

Präzipitieren Sie die eluierte Plasmid DNA durch Zugabe von **Isopropanol**, wobei der Alkohol Raumtemperatur haben sollte. Mischen Sie sofort gründlich durch Vortexen und lassen Sie die Mischung für **2 Minuten** stehen.

Zentrifugieren Sie bei **≥ 5,000 x g** für **≥15 min** bei **≤ Raumtemperatur,** vorzugsweise bei **15,000 x g** für **30 min** und **4°C**. Dekantieren Sie vorsichtig den Überstand.

3.5 ml	10.5 ml

Midi	Maxi

14 Waschen und Trocknen des DNA Pellets

Waschen Sie das DNA Pellet mit ebenfalls **Raumtemperatur** warmem, **70%igem Ethanol** und zentrifugieren Sie bei **≥ 5,000 x g**, vorzugsweise **≥ 15,000 x g** für **5 min** bei **Raumtemperatur** (20-25°C).

2 ml	5 ml

Entfernen Sie das Ethanol vollständig mit Hilfe einer Pipette. Lassen Sie das Pellet bei **Raumtemperatur** (20-25°C) an der Luft trocknen.

Hinweis: Zu langes Trocknen des Pellets kann das anschließende Lösen der DNA erschweren.

5-10 min	10-15 min

15 Lösen der DNA

Lösen Sie das DNA Pellet in einem geeigneten Volumen TE-Puffer oder in sterilem deionisiertem H_2O. Je nach Art des verwendeten Zentrifugationsgefäßes sollte die DNA unter vorsichtigem Auf- und Abpipettieren oder unter gleichmäßigem Schütteln in einem ausreichenden Volumen Puffer für 10-60 Min (3D-Schüttler) erfolgen.

Bestimmen Sie photometrisch die Plasmidausbeute und überprüfen Sie die Plasmidintegrität mittels Agarosegel-Elektrophorese (siehe Kapitel 4.11).

7.3 High-copy plasmid purification (Midi, Maxi) - French

Midi | **Maxi**

1 Préparation d'une pré-culture

Inoculez 3-5 ml d'un milieu de pré-culture LB avec une colonie piquée sur une plaque d'Agar fraîchement striée. Assurez-vous que la plaque et le milieu de culture contiennent le bon antibiotique afin d'être sûr d'obtenir le plasmide (pour d'autres informations voir section 4.3). Agitez à 37 °C à ~300 rpm pendant ~8 h.

2 Préparation d'une culture overnight

Inoculez une culture overnight en diluant la pré-culture au 1/1000ième dans un volume donné de milieu LB contenant l'antibiotique sélectif approprié. Si la culture présente une faible croissance ou si le plasmide ne se comporte pas comme un plasmide high-copy, consultez la section 4.4 du protocole pour l'utilisation de plus grands volumes de culture.

Faites pousser la culture toute la nuit à 37°C à ~300 rpm pendant 12–16 h.

Remarque : Afin d'utiliser au maximum la capacité de fixation des colonnes NucleoBond® Xtra il est nécessaire de charger une grande quantité d'ADN plasmidique. Si vous n'êtes pas sûr du nombre de copies de votre plasmide ou du comportement de la culture vis à vis de la croissance bactérienne, augmentez le volume de culture et décidez à l'étape 3 du nombre de cellules à utiliser pour la préparation.

100 ml | **300 ml**

3 Récupération des cellules bactériennes

Mesurez la DO_{600} de la culture cellulaire et déterminez le volume de culture recommandé.

V [ml] = 400 / OD_{600} | **V [ml] = 1200 / OD_{600}**

Culottez les cellules par centrifugation à **4,500 - 6,000 x g** pendant **≥10 min** à **4°C** et éliminez complètement le surnageant.

Remarque : Il est possible, bien sûr, d'utiliser de plus grands volumes, par ex. si le plasmide ne se comporte pas comme un vecteur high-copy (voir section 4.4 pour plus d'informations). Dans ces conditions il est nécessaire d'augmenter proportionnellement les volumes des tampons RES, LYS et NEU dans les étapes 4, 5 et 7. Si le volume de culture est plus du double du volume recommandé, il est plus judicieux de centrifuger pour clarifier le lysat (étape 8), plutôt que d'utiliser le filtre NucleoBond® Xtra.

Midi	Maxi

4 Resuspension

Reprendre complètement le culot cellulaire dans le tampon de resuspension **resuspension buffer RES + RNase A** en pipetant les cellules par aspiration - refoulement.

Important: pour une lyse efficace des cellules il ne doit plus subsister d'agrégats en suspension

Remarque : Augmentez proportionnellement le volume du Tampon RES si vous avez utilisé une masse cellulaire supérieure à celle recommandée (voir la section 4.6 pour plus d'informations sur les conditions de lyse optimales des cellules et la section 4.7 pour les souches difficiles à lyser).

8 ml	12 ml

5 Lyse cellulaire

Avant d'utiliser le tampon LYS vérifiez que le SDS n'a pas précipité. Si un précipité blanc est visible, chauffez le tampon quelques minutes à 30-40°C jusqu'à ce que le précipité soit complètement dissout. Ramenez le tampon à température ambiante (20-25°C).

Rajoutez le tampon de lyse **lysis buffer LYS** à la suspension.

Mélangez avec précaution en **inversant** le tube **5 fois**. Ne pas utiliser de vortex, sinon l'ADN chromosomique se fractionnerait, se détacherait des débris cellulaires et contaminerait alors la suspension.

Incubez le mélange à température ambiante (20-25°C) pendant **5 min**.

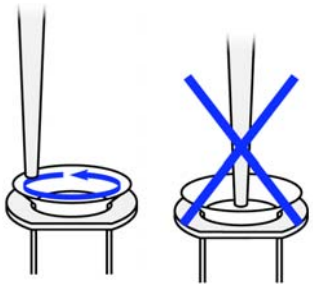
Remarque : Augmentez proportionnellement le volume du Tampon LYS si vous avez utilisé une masse cellulaire supérieure à celle recommandée (voir section 4.6 pour plus d'informations sur la lyse optimale des cellules)

8 ml	12 ml

Midi	Maxi

6 Equilibration

Equilibrez la colonne NucleoBond® Xtra **avec** le filtre intégré avec le tampon d'équilibration **equilibration buffer EQU.**



Déposez le tampon sur la collerette du filtre comme le montre le schéma. Laissez la colonne se vider par gravité et assurez-vous que le filtre est entièrement mouillé.

Ne pas laisser la colonne s'assécher.

12 ml	25 ml

7 Neutralisation

Ajoutez le tampon de neutralisation **neutralization buffer NEU** à la suspension et mélangez immédiatement avec précaution le lysat en **inversant** le tube **10-15 fois**. **Ne pas utiliser de vortex.**

Le flacon ou le tube utilisé pour cette étape ne doit pas être rempli au plus des deux tiers afin de permettre un mélange homogène. Veillez à neutraliser complètement pour précipiter toutes les protéines et l'ADN chromosomique. Le lysat doit passer d'une forme gluante et visqueuse à une forme moins visqueuse, une suspension homogène et un floculat blanc.

Procédez immédiatement à l'étape 8. **Une incubation du lysat n'est pas nécessaire**

Remarque : Augmentez proportionnellement le volume du Tampon NEU si vous avez utilisé une masse cellulaire supérieure à celle recommandée (voir section 4.6 pour des informations sur la lyse optimale des cellules)

8 ml	12 ml

Midi	Maxi

8 Clarification et chargement

Assurez-vous d'avoir une suspension homogène du précipité en **inversant le tube 3 fois** avant d'appliquer directement le lysat sur le filtre NucleoBond® Xtra préalablement équilibré, afin d'éviter que le filtre ne se bouche.

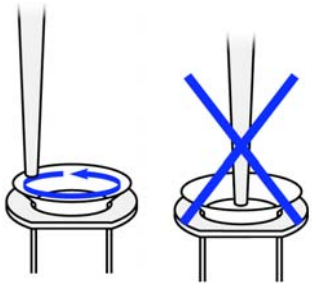
Le lysat est simultanément clarifié et chargé sur la colonne. Rechargez le filtre si le volume de lyse est plus important que la capacité du filtre. Laissez la colonne se vider par gravité.

Alternative : Le précipité peut être éliminé par centrifugation ≥5,000 x g pendant au moins 10 mn, par ex. si plus du double de la masse cellulaire recommandée a été utilisé. S' il reste des matières en suspension, transférez dans un nouveau tube et répétez l'étape de centrifugation, de préférence à une vitesse plus élevée, ou chargez le lysat sur le filtre NucleoBond® Xtra préalablement équilibré.

Cette étape de clarification est extrêmement importante. En effet, les résidus du précipité peuvent boucher la colonne NucleoBond® Xtra. Pour charger la colonne vous pouvez appliquer le lysat clarifié sur le filtre préalablement équilibré ou enlever préalablement le filtre s'il est inutilisé. Laissez la colonne se vider par gravité.

Remarque: Vous pouvez garder tous les filtrats pour les analyser (voir section 9.1)

9 Lavage du filtre et de la colonne (equilibration buffer EQU)

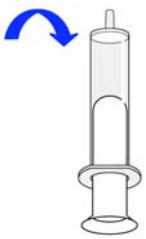


Lavez le filtre NucleoBond® Xtra et la colonne NucleoBond® Xtra avec le tampon **equilibration buffer EQU.**

Déposez le tampon sur la collerette du filtre et assurez-vous que le lysat est bien lavé. Omettre cette étape ou déposer simplement le tampon dans le filtre réduit le rendement d'ADN plasmidique.

5 ml	15 ml

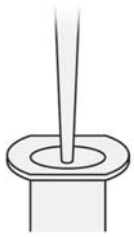
10 Eliminer le filtre



Retirez le filtre NucleoBond® Xtra ou éliminez le en retournant la colonne.

Midi	Maxi

11 Lavage de la colonne (washing buffer WASH)



Lavez la colonne NucleoBond® Xtra avec le tampon de lavage **washing buffer WASH**. Il est important d'éliminer le filtre avant d'appliquer le tampon de lavage. En effet, ceci pourrait avoir un mauvais impact sur la pureté de l'ADN.

8 ml	25 ml

12 Elution

Eluez l'ADN plasmidique avec le tampon d'élution **elution buffer ELU**.

Passez à **l'étape 13** pour le protocole de centrifugation après la précipitation à l' isopropanol ou continuez avec **la section 8** pour la concentration de l'ADN plasmidique et son désalage au moyen du système NucleoBond® Finalizer (NucleoBond® Xtra Midi Plus) ou NucleoBond® Finalizer Large (NucleoBond® Xtra Maxi Plus).

Option: Déterminez le rendement d'ADN plasmidique par spectrophotométrie UV afin d'ajuster la concentration de l'ADN à l'étape 15 et calculez le taux de récupération après l'étape de précipitation.

5 ml	15 ml

13 Précipitation

Ajoutez **l'isopropanol à température ambiante** pour précipiter l'ADN plasmidique élué. Vortexez et laissez le mélange reposer pendant **2 minutes**.

Centrifugez à **≥ 5,000 x g** pendant **≥15 min** à **≤ température ambiante,** de préférence à **15,000 x g** pendant **30 min** à **4°C**. Eliminez précautionneusement le surnageant.

3.5 ml	10.5 ml

14 Lavage et séchage du culot d'ADN

Ajoutez **l'éthanol 70% à température ambiante** sur les culots et centrifugez à ≥ 5,000 x g, de préférence ≥ 15,000 x g pendant 5 min à température ambiante (20-25°C).

2 ml	5 ml

Midi	Maxi

Eliminez précautionneusement l'éthanol complètement avec une pipette. Laissez sécher le culot à **température ambiante** (20-25°C).

Remarque: l'ADN plasmidique peut être plus difficile à dissoudre si le culot est très sec.

5-10 min	10-15 min

15 Reconstitution de l'ADN

Dissoudre le culot d'ADN dans un volume adéquat de tampon TE ou d'eau distillée stérile. En fonction du type de tube utilisé pour la centrifugation, dissoudre par aspiration - refoulement ou par rotation constante dans un volume approprié de tampon pendant 10-60 min (3D-shaker).

Déterminez le rendement d'ADN plasmidique par spectrophotométrie UV. Confirmez l'intégrité du plasmide par électrophorèse sur gel d'agarose (voir la section 4.11).

7.4 Low-copy plasmid purification (Midi, Maxi) - English

The lysis buffer volumes provided in the kit are adjusted for high-copy plasmid purification. Therefore, additional buffer has to be ordered separately for routine purification of low-copy plasmids (see section 9.2 for ordering information).

Midi	Maxi

1 Prepare a starter culture

Inoculate a 3-5 ml starter culture of LB medium with a single colony picked from a freshly streaked agar plate. Make sure that plate and liquid culture contain the appropriate selective antibiotic to guarantee plasmid propagation (see section 4.3 for more information). Shake at 37°C and ~300 rpm for ~8 h.

2 Prepare a large overnight culture

Inoculate an overnight culture by diluting the starter culture 1/1000 into the given volumes of LB medium also containing the appropriate selective antibiotic. If the culture is known to grow poorly, consult section 4.5 for larger culture volumes.

Grow the culture overnight at 37°C and ~300 rpm for 12–16 h.

Note: To utilize the entire large binding capacity of the NucleoBond® Xtra columns it is important to provide enough plasmid DNA. For the standard low-copy procedure the culture volumes were doubled compared to the high-copy vector protocol. However, due to a plasmid content that is 10-100 times lower, this might be insufficient. If you need large amounts of low-copy plasmids, further increase the culture volume by factor 3-5 (see section 4.5 for more information) and decide in step 3 how much cells to use for the preparation.

200 ml	600 ml

3 Harvest bacterial cells

Measure the cell culture OD_{600} and determine the recommended culture volume

$V\ [ml] = 800 / OD_{600}$	$V\ [ml] = 2400 / OD_{600}$

Pellet the cells by centrifugation at **4,500 - 6,000 x g** for ≥**10 min** at **4°C** and discard the supernatant completely.

Note: It is of course possible to use larger culture volumes, e. g. if a large amount of low-copy plasmid is needed (see section 4.5 for more information). In this case increase RES, LYS and NEU buffer volumes proportionally in steps 4, 5 and 7 and use a centrifuge for the lysate clarification rather than the NucleoBond® Xtra column filters.

Midi	Maxi

4 Resuspension

Resuspend the cell pellet completely in **resuspension buffer RES + RNase A** by pipetting up and down or vortexing the cells.

For an efficient cell lysis it is important that no clumps remain in the suspension.

Note: Increase RES buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis and section 4.7 regarding difficult-to-lyse strains).

16 ml	24 ml

5 Cell lysis

Check lysis buffer LYS for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30-40°C until precipitate is dissolved completely. Cool buffer down to room temperature (20-25°C).

Add **lysis buffer LYS** to the suspension.

Mix gently by **inverting** the tube **5 times**. **Do not vortex** as this will shear and release contaminating chromosomal DNA from cellular debris into the suspension.

Incubate the mixture at room temperature (20-25°C) for **5 min**.

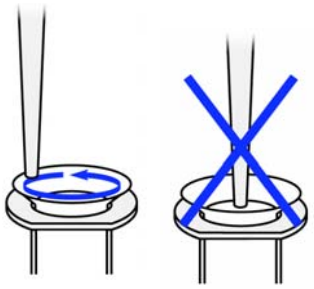
Note: Increase LYS buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis).

16 ml	24 ml

6 Equilibration

Equilibrate a NucleoBond® Xtra column together with the inserted column filter with **equilibration buffer EQU**.

Apply the buffer onto the rim of the column filter as shown in the picture. Allow the column to empty by gravity flow and make sure to wet the entire filter.



The column does not run dry.

12 ml	25 ml

Midi	Maxi

7 Neutralization

Add **neutralization buffer NEU** to the suspension and immediately mix the lysate gently by **inverting** the tube **10-15 times**. **Do not vortex.**

The flask or tube used for this step should not be filled more than two thirds to allow homogeneous mixing. Make sure to neutralize completely to precipitate all the protein and chromosomal DNA. The lysate should turn from a slimy, viscous consistency to a low viscosity, homogeneous suspension of an off-white flocculate.

Immediately proceed with step 8 of the high-copy plasmid purification protocol, section 7.1. **An incubation of the lysate is not necessary.**

Note: Increase NEU buffer volume proportionally if more than the recommended cell mass is used (see section 4.6 for information on optimal cell lysis).

16 ml	24 ml

7.5 Low-copy plasmid purification (Midi, Maxi) - German

Die in den NucleoBond® Xtra Kits enthaltenen Lysepuffer-Volumina sind ausreichend für die Isolierung von high-copy Plasmiden. Für die Isolierung von low-copy Plasmiden ist zusätzlicher Puffer notwendig (Bestellinformation siehe Kapitel 9.2).

Midi	Maxi

1 Ansetzen einer Vorkultur

Beimpfen Sie 3-5 ml LB Medium mit einer einzelnen Kolonie einer frisch ausgestrichenen Agarplatte. Stellen Sie sicher, dass sowohl die Platte als auch das Flüssigmedium das nötige Antibiotikum enthält, da bei fehlendem Selektionsdruck die Bakterien ihr Plasmid bei der Zellteilung verlieren können (für weitere Informationen siehe Kapitel 4.3). Schütteln Sie die Vorkultur bei 37°C und ~300 rpm für ~8 h.

2 Ansetzen einer Übernachtkultur

Beimpfen Sie LB Medium des unten angegebenen Volumens durch Verdünnen der Vorkultur um den Faktor 1/1000. Stellen Sie sicher, dass das Medium das nötige Antibiotikum enthält. Wählen Sie ein größeres Volumen (Kapitel 4.4), falls die Kultur bekanntermaßen langsam oder schlecht wächst.

Inkubieren Sie auf einem Schüttler bei 37°C und ~300 rpm für ~12–16 h.

Hinweis: Um die hohe Bindekapazität der NucleoBond® Xtra Säulen voll ausnutzen zu können ist es wichtig, ausreichend Plasmid DNA zu laden. Für die Standard low-copy Prozedur werden gegenüber dem high-copy Protokoll doppelte Kulturvolumina eingesetzt. Trotzdem kann dies u. U. bei einem Plasmidgehalt, der 10-100 mal geringer ist, unzureichend sein. Falls große Mengen an low-copy Plasmiden benötigt werden, sollte das Kulturvolumen nochmals um einen Faktor 3-5 erhöht werden (für weitere Informationen siehe Kapitel 4.5) und in Schritt 3 entschieden werden, wie viele Zellen für die Präparation eingesetzt werden.

200 ml	600 ml

Midi	Maxi

3 Ernte der Bakterienzellen

Messen Sie die OD_{600} der Bakterienkultur und and bestimmen das empfohlene Kulturvolumen gemäß folgender Formel

V [ml] = 800 / OD_{600}	V [ml] = 2400 / OD_{600}

Pelletieren Sie die Zellen durch Zentrifugation bei **4,500 - 6,000 x g** für **≥10 min** bei **4°C** und entfernen Sie den Überstand quantitativ.

Hinweis: Es können auch größere Kulturvolumina verwendet werden, z.B. falls große Mengen low-copy Plasmid benötigt werden (für weitere Informationen siehe auch Kapitel 4.5). In diesem Fall erhöhen Sie die Volumina der Puffer RES, LYS und NEU proportional in den Schritten 4, 5 and 7. und verwenden in Schritt 8 zur Lysatklärung eine Zentrifuge anstelle des NucleoBond® Xtra column filters.

4 Resuspension

Resuspendieren Sie das Zellpellet vollständig in **resuspension buffer RES + RNase A** durch Auf- und Abpipettieren.

Wichtig für eine effiziente Zellyse ist, dass keine Zellklumpen in der Suspension verbleiben.

Hinweis: Erhöhen Sie das Volumen des Puffers RES proportional falls mehr als die empfohlene Zellmasse eingesetzt wird (für Informationen zur optimalen Zelllyse siehe Kapitel 4.6, für schwer zu lysierende Bakterienstämme siehe Kapitel 4.7).

16 ml	24 ml

Midi	Maxi

5 Zelllyse

Vor Gebrauch überprüfen Sie lysis buffer LYS auf ausgefallenes SDS. Sollte ein weißes Präzipitat sichtbar sein, erwärmen Sie den Puffer für einige Minuten auf 30-40°C bis das Präzipitat komplett gelöst ist. Lassen Sie den Puffer auf Raumtemperatur abkühlen (20-25°C).

Geben Sie **lysis buffer LYS** zu der Suspension. Mischen Sie vorsichtig durch **5-maliges Invertieren**. **Vortexen Sie nicht,** da dies zur Scherung der genomischen DNA und zu deren Freisetzung aus den Zelltrümmern in die Suspension führen kann.

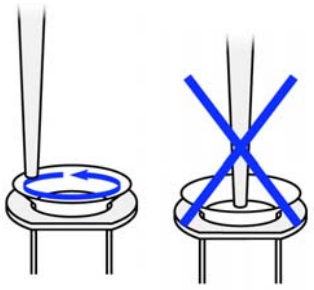
Inkubieren Sie die Mischung für **5 min** bei Raumtemperatur (20-25°C).

Hinweis: Erhöhen Sie das Volumen des Puffers LYS proportional falls mehr als die empfohlene Zellmasse eingesetzt wird (für Informationen zur optimalen Zelllyse siehe Kapitel 4.6).

16 ml	24 ml

6 Äquilibrierung

Äquilibrieren Sie eine NucleoBond® Xtra Säule zusammen mit dem eingesetzten NucleoBond® Xtra Filter mit **equilibration buffer EQU**.



Geben Sie den Puffer auf den äußeren Rand des Filters wie in der Abbildung rechts gezeigt. Lassen Sie die Flüssigkeit vollständig durch die Säule laufen und stellen Sie sicher, dass der NucleoBond® Xtra Filter komplett benetzt ist.

Die Säulen laufen nicht trocken.

12 ml	25 ml

Midi	Maxi

7 Neutralisation

Geben Sie **neutralization buffer NEU** zum Lysat und mischen Sie sofort aber vorsichtig durch **10- bis 15-maliges Invertieren**. **Vortexen Sie nicht.**

Das für diesen Schritt verwendete Gefäß sollte nicht mehr als zwei Drittel gefüllt sein um ein gleichmäßiges Durchmischen zu ermöglichen. Stellen Sie sicher, dass die Neutralisation vollständig erfolgt ist, um eine quantitative Fällung von Protein und genomischer DNA zu gewährleisten. Das schleimige, viskose Lysat sollte nach Zugabe des Puffers NEU dünnflüssig werden und eine homogene Suspension mit flockigem weißem Präzipitat ausbilden.

Fahren Sie sofort mit Kapitel 7.2, Schritt 8 des high-copy Plasmid Protokolls fort. **Eine Inkubation des Lysates ist nicht notwendig.**

Hinweis: Erhöhen Sie das Volumen des Puffers NEU proportional falls mehr als die empfohlene Zellmasse eingesetzt wird (für Informationen zur optimalen Zelllyse siehe Kapitel 4.6).

16 ml	24 ml

7.6 Low-copy plasmid purification (Midi, Maxi) - French

Midi | **Maxi**

1 Préparation d'une pré-culture

Inoculez 3-5 ml d'un milieu de pré-culture LB avec une colonie piquée sur une plaque d'Agar fraîchement striée. Assurez-vous que la plaque et le milieu de culture contiennent le bon antibiotique afin d'être sûr d'obtenir le plasmide (pour d'autres informations voir sections 4.3). Agitez à 37 °C à ~300 rpm pendant ~8 h.

2 Préparation d'une culture overnight grand volume

Inoculez une culture overnight en diluant la pré-culture au 1/1000ième dans un volume donné de milieu LB contenant l'antibiotique sélectif approprié. Si la culture présente une faible croissance ou si le plasmide est connu pour être faiblement représenté, consultez la section 4.5 du protocole pour l'utilisation de plus grands volumes de culture

Faites pousser la culture toute la nuit à 37°C à ~300 rpm pendant 12–16 h.

Remarque : Afin d'utiliser au maximum la capacité de fixation des colonnes NucleoBond® Xtra il est nécessaire de charger une quantité suffisante d'ADN plasmidique. Pour le protocole low-copy standard, le volume de culture recommandé est doublé par rapport au protocole high-copy. Cependant, étant donné que le contenu plasmidique des cellules peut être 10-100 fois inférieur, le volume de culture peut être insuffisant. Si vous avez besoin de grandes quantités de plasmides low-copy , augmentez à nouveau le volume de culture d'un facteur 3-5 (voir la section 4.5 pour plus d'informations) et décidez à l'étape 3 de la quantité de cellules à utiliser pour la préparation.

200 ml | **600 ml**

3 Récupération des cellules bactériennes

Mesurez la DO_{600} de la culture cellulaire et déterminez le volume de culture recommandé.

V [ml] = 800 / OD_{600} | **V [ml] = 2400 / OD_{600}**

Culottez les cellules par centrifugation à **4,500 - 6,000 x g** pendant ≥**10 min** à **4°C** et éliminez le surnageant complètement.

Remarque: il est possible, bien sûr, d'utiliser de plus grands volumes de culture, par ex. si un grande quantité de plasmides low-copy doit être récupérée, (voir la partie 4.5 pour plus d'informations). Dans ce cas, augmentez les volumes des tampons RES, LYS et NEU proportionnellement aux étapes 4, 5 et 7 et utilisez la centrifugation pour la clarification du lysat plutôt que les colonnes filtres NucleoBond® Xtra column filters.

Midi	Maxi

4 Resuspension

Rependre complètement le culot cellulaire dans le tampon de resuspension **re-suspension buffer RES + RNase A** en pipettant par aspiration-refoulement ou en vortexant.

Important: pour une lyse efficace des cellules il ne doit plus subsister d'agrégats en suspension

Remarque : augmentez proportionnellement le volume de tampon RES si vous avez utilisé une masse cellulaire supérieure à celle recommandée (voir la section 4.6 pour plus d'informations sur les conditions de lyse optimales des cellules et la section 4.7 pour les souches difficiles à lyser).

16 ml	24 ml

5 Lyse cellulaire

Avant d'utiliser le tampon LYS vérifiez que le SDS n'a pas précipité. Si un précipité blanc est visible, chauffer le tampon quelques minutes à 30-40°C jusqu'à ce que le précipité soit complètement dissout. Ramenez le tampon à température ambiante (20-25°C).

Ajoutez le tampon de lyse **lysis buffer LYS** à la suspension.

Mélangez avec précaution en **inversant** le tube **5 fois**. **Ne pas utiliser de vortex**, sinon l'ADN chromosomique se fractionnerait, se détacherait des débris cellulaires et contaminerait alors la suspension.

Incubez le mélange à température ambiante (20-25°C) pendant **5 min**.

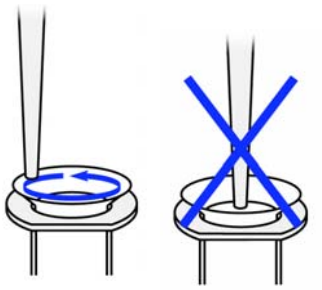
Remarque : augmentez proportionnellement le volume de tampon LYS si vous avez utilisé une masse cellulaire supérieure à celle recommandée (voir la section 4.6 pour plus d'informations sur la lyse optimale des cellules).

16 ml	24 ml

Midi	Maxi

6 Equilibration

Equilibrez la colonne NucleoBond® Xtra **avec** le filtre intégré avec le tampon d'équilibration **equilibration buffer EQU.**



Déposez le tampon sur la collerette du filtre comme le montre le schéma. Laissez la colonne se vider par gravité et assurez vous que le filtre est entièrement mouillé.

Ne pas laisser la colonne s'assécher.

12 ml	25 ml

7 Neutralisation

Ajoutez le tampon de neutralisation **neutralization buffer NEU** à la suspension et mélangez immédiatement avec précaution le lysat en **inversant** le tube **10-15 fois**. **Ne pas utiliser de vortex.**

Le flacon ou le tube utilisé pour cette étape ne doit pas être rempli au plus des deux tiers afin de permettre un mélange homogène. Veillez à neutraliser complètement pour précipiter toutes les protéines et l'ADN chromosomique. Le lysat doit passer d'une forme gluante et visqueuse à une forme moins visqueuse, une suspension homogène et un floculat blanc.

Passez à l'étape 8 du protocole de purification des plasmides high-copy, section 7.1. **Une incubation du lysat n'est pas nécessaire.**

Remarque : augmentez proportionnellement le volume de tampon NEU si vous avez utilisé une masse cellulaire supérieure à celle recommandée (voir la section 4.6 pour plus d'informations sur la lyse optimale des cellules).

16 ml	24 ml

8 Concentration of NucleoBond® Xtra eluates with the NucleoBond® Finalizers

The following section includes the protocols for concentration of NucleoBond® Xtra eluates with the NucleoBond® Finalizers in English, German, and French.

8.1 Concentration of NucleoBond® Xtra eluates with the NucleoBond® Finalizers - English

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

1 Precipitate DNA

Add **0.7 volumes** of **room-temperature isopropanol** (not supplied with the kit). Vortex well and let the mixture sit for **2 minutes**.

(e.g. for 5 ml NucleoBond® Xtra Midi eluate add **3.5 ml** isopropanol, for 15 ml NucleoBond® Xtra Maxi eluate add **10.5 ml** isopropanol)

*Note: The **NucleoBond® Finalizer** only holds up to 500 µg and the **NucleoBond® Finalizer Large** is limited to 2000 µg of plasmid DNA. Please check plasmid content of your eluate prior to the precipitation step by measuring A_{260} (see section 4.11). Loading more DNA might lead to clogging and complete loss of your sample.*

3.5 ml for 5 ml eluate	10.5 ml for 15 ml eluate

2 Load precipitate

Remove the plunger from a 30 ml syringe and attach a NucleoBond® Finalizer to the outlet. Fill the precipitation mixture into the syringe, insert the plunger and press the mixture slowly through the NucleoBond® Finalizer using constant force. Discard the flow-through.

3 Wash precipitate

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

Fill **2 ml** of **70 % ethanol** (not supplied with the kit) into the syringe, insert the plunger and press the ethanol slowly through the NucleoBond® Finalizer. Discard the ethanol.

2 ml	2 ml

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

4 Dry filter membrane

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer. Press air through the NucleoBond® Finalizer with appropriate force while touching a tissue with the tip of the NucleoBond® Finalizer to soak up ethanol.

Repeat this step at least two times until no more ethanol leaks from the NucleoBond® Finalizer.

Note: A new dry syringe can be used to speed up the procedure (not provided).

Optional: You can incubate the NucleoBond® Finalizer for 10 minutes at 80°C to minimize ethanol carry-over. However, the final recovery may be reduced by over-drying the DNA.

5 Elute DNA

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger of a **1 ml syringe** and attach the NucleoBond® Finalizer to the syringe outlet.

Note: Refer to section 4.9, Figures 3 (Midi) or 4 (Maxi) to choose the appropriate volume of elution buffer.

Pipette 500-1000 µl of **redissolving buffer TRIS** (5 mM Tris/HCl, pH 8.5) or TE buffer into the syringe (see section 4.10). Place the NucleoBond® Finalizer outlet over a fresh collecting tube and **elute plasmid DNA** carefully by inserting the plunger.

500-1000 µl	500-1000 µl

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

Transfer the first eluate back into the syringe and elute into the same collecting tube a second time.

load first eluate completely	load first eluate completely

6 Determination of yield

Determine plasmid yield by UV spectroscopy and confirm plasmid integrity by agarose gel electrophoresis (see section 4.11).

8.2 Concentration of NucleoBond® Xtra eluates with the NucleoBond® Finalizers – German

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

1 Präzipitation

Präzipitieren Sie die eluierte Plasmid DNA durch Zugabe von **0.7 Volumen Isopropanol** (nicht im Lieferumfang enthalten), wobei der Alkohol **Raum-temperatur** haben sollte. Mischen Sie sofort gründlich durch Vortexen und lassen Sie die Mischung für **2 Minuten** stehen.

(Geben Sie z. B. **3.5 ml** Isopropanol zu 5 ml NucleoBond® Xtra Midi Eluat oder **10.5 ml** Isopropanol zu 15 ml NucleoBond® Xtra Maxi Eluat)

*Hinweis: Die Bindekapazität des **NucleoBond® Finalizers** beträgt 500 µg Plasmid DNA, die des **NucleoBond® Finalizer Large** beträgt 2000 µg. Überprüfen Sie den Plasmidgehalt des Eluates vor der Präzipitation durch Bestimmung des A_{260} (siehe Kapitel 4.11). Die Beladung mit größeren DNA Mengen kann zum Verstopfen des **NucleoBond® Finalizers** und somit zum kompletten Verlust Ihrer Probe führen.*

3.5 ml für 5 ml Eluat	10.5 ml für 15 ml Eluat

2 Laden des Präzipitats

Ziehen Sie den Kolben einer 30 ml Spritze vollständig heraus und befestigen Sie den NucleoBond® Finalizer am Auslass der Spritze.

Füllen Sie das Präzipitationsgemisch in die Spritze, setzen Sie den Kolben ein und drücken Sie die Suspension langsam mit konstantem Druck durch den NucleoBond® Finalizer. Verwerfen Sie den Durchfluss.

3 Waschen des Präzipitats

Entfernen Sie den NucleoBond® Finalizer von der Spritze, ziehen Sie den Kolben heraus und befestigen Sie den NucleoBond® Finalizer wieder am Auslass der Spritze.

Füllen Sie **2 ml 70 %iges Ethanol** (nicht im Lieferumfang enthalten) in die Spritze, setzen Sie den Kolben ein und drücken Sie das Ethanol langsam durch den NucleoBond® Finalizer. Verwerfen Sie das Ethanol.

2 ml	2 ml

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

4 Trocknen der Filtermembran

Entfernen Sie den NucleoBond® Finalizer von der Spritze, ziehen Sie den Kolben heraus und befestigen Sie den NucleoBond® Finalizer wieder am Auslass der Spritze. Drücken Sie so kräftig wie möglich Luft durch den NucleoBond® Finalizer und nehmen das an der Spitze austretende Ethanol mit einem Tuch auf. Wiederholen Sie diesen Schritt mindestens zweimal bis kein Ethanol mehr aus dem NucleoBond® Finalizer austritt.

Hinweis: Zur Beschleunigung der Prozedur kann eine neue trockene Spritze verwendetet werden (nicht im Lieferumfang enthalten).

Optional: Um die Ethanol-Verschleppung ins Eluat zu minimieren, inkubieren Sie den NucleoBond® Finalizer für 10 Minuten bei 80°C. Zu intensives Trocknen der DNA kann allerdings zu einer reduzierten Wiederfindung führen..

5 Elution der DNA

Entfernen Sie den NucleoBond® Finalizer von der 30 ml Spritze, ziehen Sie den Kolben einer **1 ml Spritze** heraus und befestigen den NucleoBond® Finalizer am Auslass der Spritze.

Hinweis: Zur Wahl des geeigneten Elutionspuffer-Volumens siehe Kapitel 4.9, Abbildung 3 (Midi) oder 4 (Maxi).

Pipettieren Sie **redissolving buffer TRIS** (5 mM Tris/HCl, pH 8.5, im Lieferumfang enthalten) oder TE Puffer in die Spritze (siehe auch Kapitel 4.10). Platzieren Sie den Auslass des NucleoBond® Finalizers über einem frischen Auffanggefäß und **eluieren Sie die Plasmid DNA langsam**.

500-1000 µl	500-1000 µl

Entfernen Sie den NucleoBond® Finalizer von der Spritze, ziehen Sie den Kolben heraus und befestigen den NucleoBond® Finalizer wieder am Auslass der Spritze.

Überführen Sie das erste Eluat zurück in die Spritze und eluieren ein zweites mal in das selbe Auffanggefäß.

erstes Eluat vollständig laden	erstes Eluat vollständig laden

Midi - NucleoBond® Finalizer

Maxi - NucleoBond® Finalizer Large

6 Bestimmung der Plasmid-Ausbeute

Bestimmen Sie photometrisch die Plasmidausbeute und überprüfen Sie die Plasmidintegrität mittels Agarosegel-Elektrophorese (siehe Kapitel 4.11).

8.3 Concentration of NucleoBond® Xtra eluates with the NucleoBond® Finalizers – French

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

1 Précipitation de l'ADN

Ajoutez **0.7 volumes d'isopropanol à température ambiante** (non fourni dans les kits). Vortexez précautionneusement et laissez le mélange reposer pendant **2 minutes**.

(ex. pour 5 ml d'éluat NucleoBond® Xtra Midi ajoutez **3.5 ml** d'isopropanol, pour 15 ml d'éluat NucleoBond® Xtra Maxi, ajoutez **10.5 ml** d'isopropanol)

*Remarque : le **NucleoBond® Finalizer** peut retenir au maximum jusqu'à 500 µg et le **NucleoBond® Finalizer Large** est limité à 2000 µg d'ADN plasmidique. Vérifier le contenu d'ADN plasmidique de vos éluats avant de passer à l'étape de précipitation en mesurant A_{260} (voir la section 4.11). Charger plus d'ADN peut entraîner une surcharge et une perte complète de l'échantillon.*

3.5 ml pour 5 ml d'éluat	10.5 ml pour 15 ml d'éluat

2 Chargement du précipité

Enlevez le piston de la seringue 30 ml et fixez un NucleoBond® Finalizer en sortie. Versez le mélange de précipitation dans la seringue, insérez le piston et pressez le mélange afin de le faire passer doucement à travers le NucleoBond® Finalizer en utilisant une force constante. Eliminez le filtrat.

3 Lavage du précipité

Enlevez le NucleoBond® Finalizer de la seringue, retirez le piston et rattachez le NucleoBond® Finalizer à la sortie de la seringue.

Versez **2 ml d'éthanol 70%** (non fourni dans les kits) dans la seringue, insérez le piston, et pressez l'éthanol doucement à travers le NucleoBond® Finalizer. Eliminez l'éthanol.

2 ml	2 ml

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

4 Séchage de la membrane

Enlevez le NucleoBond® Finalizer de la seringue, retirez le piston et rattachez le NucleoBond® Finalizer. Faites passer de l'air à travers le NucleoBond® Finalizer avec une force appropriée, tout en touchant un tissus ou papier propre avec la pointe du NucleoBond® Finalizer pour absorber l'éthanol.

Répétez cette étape au moins deux fois jusqu'à ce que plus aucune goutte d'éthanol ne sorte du NucleoBond® Finalizer.

Remarque : une nouvelle seringue sèche peut être utilisée pour accélérer la procédure (non fournie).

Option: vous pouvez incuber NucleoBond® Finalizer pendant 10 minutes à 80°C pour minimiser la contamination éthanolique. Cependant, le taux de récupération final peut être réduit en raison d'un séchage excessif de l'ADN.

5 Elution de l'ADN

Enlevez le NucleoBond® Finalizer de la seringue, retirez le piston d'une **seringue 1 ml** et fixez le NucleoBond® Finalizer à la sortie.

Remarque : voir la section 4.9, Figures 3 (Midi) ou 4 (Maxi) pour choisir le volume approprié de tampon d'élution.

Pipetez 500-1000 µl de tampon d'élution **buffer TRIS** (5 mM Tris/HCl, pH 8.5 , fourni dans le kit) ou le tampon TE (voir la section 4.10) dans la seringue. Placez la pointe du NucleoBond® Finalizer au dessus d"un nouveau tube collecteur et **éluez l'ADN plasmidique** précautionneusement en insérant le piston.

500-1000 µl	500-1000 µl

Enlevez le NucleoBond® Finalizer de la seringue, retirez le piston et rattachez le NucleoBond® Finalizer à la sortie de la seringue.

Transférez le premier éluat dans la seringue et éluez dans le même tube collecteur une seconde fois.

Charger complètement le premier éluat	Charger complètement le premier éluat

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

6 Détermination du rendement

Déterminez le rendement d'ADN plasmidique par spectrophotométrie UV et confirmez l'intégrité du plasmide par électrophorèse sur gel d'agarose (voir section 4.11).

9 Appendix

9.1 Troubleshooting

If you experience problems with reduced yield or purity, it is recommended to check which purification step of the procedure is causing the problem.

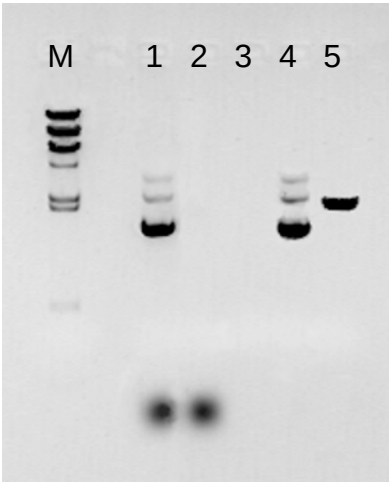
First, the bacterial culture has to be checked for sufficient growth (OD_{600}) in the presence of an appropriate selective antibiotic (Table 1). **Second**, aliquots of the cleared lysate, the flow-through, the combined washing steps (buffers EQU and WASH), and the eluate should be kept for further analysis by agarose gel electrophoresis.

Refer to Table 4 to choose a fraction volume yielding approximately 5 µg of plasmid DNA. The volumes outlined in Table 4 refer to maximum yield per binding capacity of each column size used for the preparation. Precipitate the nucleic acids by adding 0.7 volumes of isopropanol, centrifuge the sample, wash the pellet using 70% ethanol, centrifuge again, air dry for 10 minutes, dissolve the DNA in 100 µl TE buffer, pH 8.0, and run 20 µl on a 1% agarose gel. The gel picture (Figure 5) will help you to address the specific questions outlined in this section more quickly and efficiently.

Table 4: NucleoBond® Xtra eluate volumes required for an analytical check

Sample	Purification step	Volume required [µl]	
		Midi	Maxi
I	**Cleared lysate, of protocol step 8**	500	200
II	**Column flow-through, after protocol step 8**	500	200
III	**Wash flow-through, after protocol step 9 and 11**	250	200
IV	**Eluate, after protocol step 12**	100	100

Figure 5 **Analytical check of NucleoBond® Xtra Midi purification samples**
Plasmid: pUC18, bacterial strain: *E.coli* DH5α. 20 µl of each precipitated sample has been analyzed on a 1% agarose gel. Equal amounts of plasmid DNA before (lane 1) and after (lane 4) purification using NucleoBond® Xtra Midi are shown with a recovery of > 90%.



M: Marker λ *Hin*dIII
1: I, cleared lysate, *ccc, linear and oc structure of the plasmid, degraded RNA*
2: II, lysate flow-through, *no plasmid DNA, but degraded RNA*
3: III, wash flow-through, *no plasmid DNA or residual RNA*
4: IV, eluate, *pure plasmid DNA*
5: *Eco*RI restriction, *linearized form of plasmid*

Problem	Possible cause and suggestions
No or low plasmid DNA yield	*Plasmid did not propagate* • Check plasmid content in the cleared lysate by precipitation of an aliquot. Use colonies from fresh plates for inoculation and add selective antibiotic to plates and media. • Estimate plasmid content prior to large purifications by a quick NucleoSpin® Plasmid or NucleoSpin® Plasmid QuickPure preparation. *Alkaline lysis was inefficient* • Too much cell mass was used. Refer to section 4.4-4.6 regarding recommended culture volumes and lysis buffer volumes. • Check buffer LYS for SDS precipitation before use, especially after storage below 20°C. If necessary incubate the bottle for several minutes at 30-40°C and mix well until SDS is redissolved. *SDS- or other precipitates are present in the sample* • Load the crude lysate onto the NucleoBond® Xtra column filter inserted in the NucleoBond® Xtra column. This ensures complete removal of SDS precipitates. Incubation of cleared lysates for longer periods of time might lead to formation of new precipitate. If precipitate is visible, it is recommended to filter or centrifuge the lysate again directly before loading it onto the NucleoBond® Xtra column. *Sample/lysate is too viscous* • Too much cell mass was used. Refer to section 4.4-4.6 regarding recommended culture volumes and lysis buffer volumes. • Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA. Otherwise, filtration efficiency and flow rate go down and SDS prevents DNA from binding to the column. *pH or salt concentrations of buffers are too high* • Keep all buffers tightly closed. Check and adjust pH of buffer LYS (pH 6.5) and NEU (pH 9.0).

Problem	Possible cause and suggestions
NucleoBond® Xtra column filter clogs during filtration	*Culture volumes are too large* • Refer to section 4.4-4.6 regarding recommended culture volumes and larger lysis buffer volumes. *Precipitate was not resuspended before loading* • Invert crude lysate at least 3 times directly before loading. *Incomplete precipitation step* • Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA.
NucleoBond® Xtra column is blocked or very slow	*Sample is too viscous* • Do NOT attempt to purify lysate prepared from a culture volume larger than recommended for any given column size with standard lysis buffer volumes. Incomplete lysis not only blocks the column but can also significantly reduce yields. Refer to section 4.4 and 4.5 for recommended culture volumes and section 4.6 for larger culture volumes and adjusted lysis buffer volumes. • Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA. *Lysate was not cleared completely* • Use NucleoBond® Xtra column filter or centrifuge at higher speed or for a longer period of time. • Precipitates occur during storage. Clear lysate again before loading the column.
Genomic DNA or RNA contamination of plasmid DNA	*Lysis treatment was too harsh* • Make sure not to lyse in buffer LYS for more than 5 min. *Lysate was mixed too vigorously or vortexed after lysis* • Invert tube for only 5 times. Do not vortex after addition of LYS. • Use larger tubes or reduce culture volumes for easier mixing. *RNase digestion was inefficient* • RNase was not added to buffer RES or stored improperly. Add new RNase to buffer RES. See section 9.2 for ordering information.

Problem	Possible cause and suggestions
Low purity (A_{260}/A_{280} < 1.8)	*NucleoBond® Xtra column filter was not removed before second washing step* • Protein content too high due to inefficient washing. Remove the NucleoBond® Xtra column filter BEFORE performing the second washing step with buffer WASH. *Buffer WASH instead of buffer EQU was used for the first wash* • Buffer EQU has to be used to wash out the NucleoBond® Xtra column filter to avoid protein carryover. *Only minimal amounts of DNA were loaded onto the column* • To much free binding capacity needs more extensive washing – double washing step with buffer WASH.
White precipitate in eluate	*NucleoBond® Xtra column filter was not removed before second washing step* • Spin down the white precipitate and continue with DNA precipitation from supernatant.
Nucleic acid pellet is opaque or white instead of clear and glassy	*Co-precipitation of salt* • Check isopropanol purity, and perform precipitation at room temperature (20-25°C) but centrifuge at 4°C. Do not let the eluate drip from the column into isopropanol but add isopropanol to the final eluate and mix immediately. • Try resuspending the pellet in buffer WASH, and reload onto the same NucleoBond® Xtra column. Wash the column several times with buffer WASH before loading.

Problem	Possible cause and suggestions
No nucleic acid pellet formed after precipitation	*Pellet was lost* • Handle the precipitate with care. Decant solutions carefully. Determine DNA yield in buffer ELU in order to calculate the plasmid DNA that should be recovered after precipitation. *Plasmid DNA might be smeared over the wall of the tube* • Dissolve DNA with an appropriate volume of TE buffer by rolling the tube for at least 30 min. *Nucleic acid did not precipitate* • Check type and volumes of precipitating solvent. Make sure to use at least 0.7 volumes of isopropanol. • Centrifuge for longer periods of time at higher speed.
Nucleic acid pellet does not resuspend in buffer	*Pellet was over-dried* • Try to dissolve at higher temperatures for a longer period of time (e.g., 2 h at 37°C or overnight at RT), preferably under constant spinning (3D-shaker). *Co-precipitation of salt or residual alcohol* • Wash the pellet again with 70 % ethanol, or increase the reconstitution buffer volume.

Problem	Possible cause and suggestions
Purified plasmid does not perform well in subsequent reactions	*Plasmid DNA is contaminated with chromosomal DNA or RNA* • Refer to the detailed trouble shooting above. *Plasmid DNA is contaminated with residual alcohol* • Plasmid DNA was not dried completely before redissolving. Precipitate DNA again by adding 1/10 volume of 3 M NaAc pH 5.0 and 0.7 volumes of isopropanol. Proceed with the precipitation protocol in this manual und dry DNA Pellet completely. *DNA is degraded* • Make sure that your entire equipment (pipettes, centrifuge tubes, etc.) is clean and nuclease-free. • Do not lyse the sample with buffer LYS for more than 5 min. *DNA is irreversibly denatured.* • A denatured plasmid band runs faster on the gel than the supercoiled conformation. Do not lyse the sample after addition of LYS for more than 5 minutes.
No or low plasmid DNA yield after NucleoBond® Finalizer precipitation	*Loss of eluate too high due to dead volume* • Especially when you aim for high concentration you need to elute in small volumes. But naturally you will lose parts of your eluate in the syringe and on the NucleoBond® Finalizer. To minimize these losses in the second elution step, try to transfer even the last droplet from the syringe to the NucleoBond® Finalizer, e.g. by tapping the NucleoBond® Finalizer and syringe onto the bench top. Then fill the syringe with air and press forcefully the last droplets out of the NucleoBond® Finalizer. Repeat this step several times. You might have to practice this procedure several times to achieve optimal results. An acceptable dead volume is smaller than 30 µl with NucleoBond® Finalizer and 75 µl with NucleoBond® Finalizer Large.

Problem	Possible cause and suggestions
Low DNA concentration after NucleoBond® Finalizer precipitation (*continued*)	*Elution volume too small* • Since there are dead volumed of about 30 µl (NucleoBond® Finalizer) and 75 µl (NucleoBond® Finalizer Large), reasonable elution volumes start with 100 µl (NucleoBond® Finalizer) and 400 µl (NucleoBond® Finalizer Large). Furthermore, smaller volumes are insufficient to wet the entire membrane and will drastically decrease your yield. *Forgot to elute a second time* • Repeating the elution procedure with the first eluate is crucial for optimal yields. However, eluting a third time shows no further improvement. • Fresh elution buffer was used for second elution step • The second elution step is crucial for optimal yield but to achieve a high DNA concentration the eluate of the first elution step has to be used for the second elution. *Plasmid size* • Precipitation efficiency is almost independent of plasmid size, but elution becomes more and more difficult for larger constructs. If you face low yields with large cosmids you may try heating the NucleoBond® Finalizer, the syringes, and elution buffer to 70°C. *Already no or low plasmid DNA after elution* • Refer to detailed trouble shooting *No or low plasmid DNA yield*. *Not enough DNA loaded* • Since there is a technical limitation to at least 100 µl (NucleoBond® Finalizer) and 400 µl (NucleoBond® Finalizer Large) of elution buffer due to membrane wetting and dead volume, a minimal amount of DNA has to be loaded to achieve a desired concentration. If possible try to pool several DNA precipitation batches since percentage of recovery and concentration significantly increase with higher amounts of loaded DNA.

9.2 Ordering information

Product	Cat. No.	Pack of
NucleoBond® Xtra Midi	740410.10	10 preps
NucleoBond® Xtra Midi	740410.50	50 preps
NucleoBond® Xtra Midi	740410.100	100 preps
NucleoBond® Xtra Midi Plus	740412.10	10 preps incl. NucleoBond® Finalizer
NucleoBond® Xtra Midi Plus	740412.50	50 preps incl. NucleoBond® Finalizer
NucleoBond® Xtra Maxi	740414.10	10 preps
NucleoBond® Xtra Maxi	740414.50	50 preps
NucleoBond® Xtra Maxi	740414.100	100 preps
NucleoBond® Xtra Maxi Plus	740416.10	10 preps incl. NucleoBond® Finalizer Large
NucleoBond® Xtra Maxi Plus	740416.50	50 preps incl. NucleoBond® Finalizer Large
NucleoBond® Xtra combi rack	740415	1
NucleoBond® Xtra buffer set	740417	1
NucleoBond® Finalizer*	740519.20	20 filters, 2 syringe sets
NucleoBond® Finalizer Plus*	740520.20	20 sets, 20 syringe sets
RNase A	740505	100 mg
RNase A	740505.50	50 mg

9.3 Product use restriction / warranty

NucleoBond® Xtra Midi/Maxi kit components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

* The NucleoBond® Finalizer/Finalizer Plus kits are suitable for use with NucleoBond® Xtra Midi kits only (see section 4.9).

It is rather the responsibility of the user to verify the use of the **NucleoBond® Xtra Midi/Maxi** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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